HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON THE CYSTS OF SOME TAPEWORMS OF THE GENUS TAENIA (SENSULATO) WITH PARTICULAR REFERENCE TO THE CUTICLE

by

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INTRODUCTION

Tapeworms present many aberrant features and they are relatively difficult subjects for histological study since their tissues are syncytial, consequently, accounts of their histology and histogenesis have contained contradictory features. The conflicting items have defied resolution until very recently when the advent of the electron microscope introduced a quite new line of evidence. The confusion was especially marked in relation to the cuticle, its origin and relationship to the subcuticular cells, and to the developmental origin of these cells. In spite of the quite intense study which they had received up to that date, MacBride (1914) was compelled to omit the Cestodes and Trematodes from consideration in his textbook of Invertebrate Embryology.

Zeder (1800) first used the term cysticercus to describe a bladder worm of the dog. Bladderworms and hydatids had been known from antiquity but their relationship with tapeworms was not suspected until von Siebold (1845) drew attention to the similarity between the proscolices of bladderworms and the scolices of adult tapeworms. Küchenmeister (1852) experimentally identified the relationship and was thus the first to discover the nature of the tapeworm life-cycle. He demonstrated the life cycles of Taenia pisiformis and

T. solium and other workers rapidly elucidated the life-cycles of other species (Leuckart 1886).

The morphology of tapeworm larvae has received continuous attention from the earliest days of the last century right up to the present. Of particular importance the following may be mentioned:- Cobbold (1864), Moniez (1880), Raum (1883), Leuckart (1886), Bartels (1902), Stiles (1906), Young (1908), Viljoen (1937), Crusz (1948a, b,c), Rees (1951), McIntosh (1956), Hutchison (1958) and Voge (1962). Wardle & McLeod (1951) have given a wide review.

Young (1908) and Pratt (1909) have discussed the views of earlier workers on the vexed problem of an epithelium in tapeworms and the relation of the subcuticular cells to the original germ layers. As regards the nature of the cuticle itself the following theories have been held, each of which has had its followers:-

(1) The cuticle is a modified epithelium containing degenerated nuclei and other cellular remains (Salensky 1874 & Young 1935)

(2) The subcuticular cells are epithelial and the cuticle is their secretion (Blochman 1896)

(3) The epithelium is lacking and the cuticle is secreted by subcuticular

cells which are specialised parenchymatous elements, (Young 1908 & Pratt 1909).

Although Young (1908 argued that cestodes were so aberrant as to make comparisons between them and the other Metazoa with their distinct germ layers pointless, he reverted in 1935, after studies on Turbellaria, towards the view (1) that the cuticle of cestodes was a condensed modified epithelium. These views with their respective adherents prevailed until the last decade when the electron micrograph studies of Read (1955), Kent (1957) and Rothmann (1959 and 1960) brought forward the opinion that the cuticle is a living entity in cytoplasmic continuity with the subcuticular cells. Threadgold's (1962) work makes it very difficult not to accept this last interpretation. The embryonic relationships of this tegumentary syncytium , however, remains an open question. Electron microscope studies up to date have been concerned in all cases with adult tapeworms from the gut of the host.

Many workers have been concerned with biochemical analysis of whole tapeworms and von Brand (1952) has reviewed the findings. Of particular interest is the low relative concentration of proteins. Proteins cenapsed with various other molecules such as cerebrosides and bile-salts, and with varying proportions of glycogen were obtained from Moniezia expansa by Kent (1947) and Kent & Macheboeuf (1948). In this latter work the authors also showed that the bulk of the lipoid material was in the form of phospholipids.

At the histochemical level, chemical analysis of tapeworms is a virtually untouched subject. Almost the whole of the work carried out up to date has been concerned with the distribution of food materials, and in particular of glycogen and fats. Comparable studies have been undertaken on trematodes. These studies include those of:- Axmann (1947) on glycogen in Schistosomes: Stephenson (1947) on fats in Fasciola hepatica: von Brand (1947) on glycogen and fats on adult and larval Schistosomes: Hedrick (1957 & 1958) on glycogen and fats respectively on adults of Hymenolepis diminuta and Raillietina cesticillus: Heyneman and Voge (1957) and Voge (1960) on glycogen and fats respectively of Hymenolepidid cysticercoids.

Baer (1951) has commented on the complete lack of information covering the chemical composition of the cestode integument, which, in view of the absence of a gut, is of paramount importance to an understanding of its nutritional physiology. By that date the only information of any relevance was that of Krudenmier (1948) who showed that the cuticle of the trematode Paragonimus kellicotti contained a mucoprotein. There has been but little information added since that date on histochemistry of structural features of tapeworms. Crusz (1947) published some results on the chemical development of the hooks of Cysticercus fasciolaris. Concerning the cuticle proper, Holz and Pezenburg (1957) demonstrated

a negative Periodic acid-Schiff reaction with C. bovis cuticles, Kilejian et al (1961) found the cuticle of Echinococcus granulosus to be PA/S positive after salivary digestion. Voge (1962) using the technique of Rinehart and Abul-Haj (1951) concluded that the cuticle of Cysticercus tenuicollis contained an acid mucopolysaccharide.

The present studies were undertaken

(1) to investigate the chemical nature of the cuticle of various larval tapeworms, and the chemical development of the cuticle of Cysticercus fasciolaris at different ages, and

(2) to ascertain the relationship between larval and adult cuticles and in particular to determine how far recent electron microscopic findings on adult cuticles were applicable to those of larvae.

Note on Nomenclature

Since the Taeniid tapeworms alternate in their life-cycles between two host species they have been described under two sets of Linnean binomials applied to their adult and larval stages in carnivore and herbivore hosts respectively. The larval or bladder-worm stage has been described under the "generic" name Cysticercus, Zeder (1800). Since this term has a wide

currency among meat inspectors, veterinarians and others concerned with domestic animals and their parasites it has been employed throughout this thesis. The following larval tapeworms have been studied and they are referred to as Cysticercus together with the appropriate trivial name as follows:-

1. Taeniarhynchus saginatus (Gofze, 1782)
   Wardle & McLeod,(1952)
   (= Taenia saginata (Gofze, 1782) auctt)
   Larva Cysticercus bovis (Cobbold 1866)

2. Taenia hydatigena (Pallas, 1766)
   Larva Cysticercus tenuicollis (Rudolphi 1819)

3. Taenia pisiformis (Bloch, 1780)
   Larva Cysticercus pisiformis (Bloch, 1780)

4. Hydatigera taeniaeformis (Batsch, 1786)
   Wardle & McLeod,(1952)
   (= Taenia taeniaeformis (Batsch, 1786) auctt)
   Larva Cysticercus fasciolaris (Rudolphi 1808)

Comparative tests were made on:-

5. Taenia solium (Linnaeus, 1758)
   Larva Cysticercus cellulosae (Gmelin 1790)

6. Multiceps multiceps (Liske 1780)
   Larva Coenurus cerebralis (Batsch 1786)

MATERIALS and METHODS

The material used in these studies included: mature cysts of Cysticercus bovis, C. pisiformis, C. tenuicollis and various development stages of C. fasciolaris. Brief reference is also made to C. cellulosae and Coenurus cerebralis.

Cysticercus bovis was obtained from the masseter muscles of freshly slaughtered oxen from Edinburgh Abattoir. This species has a low incidence in Scotland (Morsden, 1950) and only a small number of cysts was, therefore, available for study. On one occasion, however, a useful collection was made from a single bullock with a generalised cysticercosis. Forty-nine viable cysts were obtained from the masseter muscle alone and others were also collected from heart, diaphragm, and neck muscles. This single animal consequently produced the major part of the material for the study of this species.

Cysticercus tenuicollis: cysts were collected from the mesenteries of sheep killed in the Edinburgh Abattoir and were abundantly available.

Fifteen cysts of Cysticercus pisiformis were generously supplied by the Parasitological Dept. of the Ministry of Agriculture, Veterinary Laboratory, Weybridge. In view of the difficulty in obtaining this species locally an attempt was made to maintain it experimentally

in the laboratory. Ten cysts were administered to a 6 month old puppy. In spite of constant surveillance only two gravid proglottides were recovered from this animal during a period of about 4 months. Finally "Nemural" was administered by the Veterinary Dept. with the aim of securing expulsion of the tapeworms. None were recovered, however, and it is assumed that the animal had already rid itself of the infestation. Two 8-weeks old rabbits were fed with one each of the segments obtained. After 10 weeks 21 and 35 bladderworms were recovered from them. Again 10 cysts each were administered to a further pair of puppies aged 6 months and 1 year but in neither of them was there a successful establishment of adult tapeworms. Further attempts to cultivate the species were abandoned and the work was carried out on the surplus 31 cysts already to hand. It had been intended to carry out studies on the development of Cysticercus pisiformis in the rabbit but the failure to infest the definitive host with the adult phase compelled the resort to another species namely Cysticercus fasciolaris. This is to be regretted, however, since this latter species undergoes strobilation in the intermediate host and hence is less directly comparable to the species C. bovis and C. tenuicollis than is Cysticercus pisiformis.

Eggs of Hydatigera taeniaeformis obtained from experimentally infested cats, and some 32 mice infested

with the intermediate stage, C. fasciolaris, were kindly supplied by the Wellcome Laboratory of Tropical Medicine, London, through the kind offices of Mr. J.W. Barber-Lomax. The eggs were used to set up infestation in mice (stain A obtained from the same source as that used by the Wellcome Laboratory, namely, W. Clarke of Oldham, Lancs.). The eggs which had been supplied in normal saline, at a rate of 2,000 eggs per ml., were fully embryonated when received. They were stored at 4°C in normal saline which was changed after centrifugation every third day, to prevent gross contamination of the material with micro-organisms. Although, Hutchinson (1958) has shown that eggs remain viable for up to 5 months under such conditions, the maximum period of egg storage used in the production of cysts for this work was 1 month. Male mice only were used and were infested at between 4 and 6 weeks of age. Approximately 100 eggs were administered to each mouse by stomach tube: polythene tubing, outside diameter 1.0 mm. connected to a number 17 hypodermic needle, was fed from a tuberculin syringe. The eggs for each dose were suspended in 1 ml. of normal saline. Mice were killed at 10 - 20 - 40 - and 60-day intervals after infestation to obtain successive stages of development of the parasite and the infested mice obtained direct from London were used to supplement the material for study of the fully-developed cyst.

A sheep's head from Kenmare, Co. Kerry, containing Multiceps multiceps was obtained from J.W. Thoma, Esq.,

M.R.C.V.S., and cysts of Cysticercus cellulosae from a pig slaughtered in Livingstone Abattoir, Northern Rhodesia were collected by Dr. J. Allan Campbell.

Bladderworms recovered in the abattoir were removed from the host while still encapsulated within the host connective-tissue capsule and were transported thus to the laboratory. They were there excapsulated and cut into small pieces immediately prior to immersion in fixative fluids. Cysticercus pisiformis was processed in the same way. The procedure differed slightly when dealing with C. fasciolaris. Ten-day old cysts are so small that they are only visible through a hand lens and with this they can be recognised at the margin of the liver. They, as well as 20-days old cysts, had to be removed along with small amounts of liver tissue and fixed without further separation. Although forty-day old cysts are readily removed from the liver tissue they are not readily excapsulated. They were, therefore, fixed as entire encapsulated cysts. By 60 days the parasite is fully grown and can now be excapsulated: they were treated, therefore, in the same manner as the mature abattoir specimens of the other species.

Since the choice of fixative, method of embedding etc. were determined by the process or test to which the samples were to be submitted, the methods used are described seriatim under the headings Histology, Electron Microscopy, and Histochemistry.

Histology: For histological examination of the 4 principal species comparisons were made between the fixative solutions of Zenker, Carnoy, Helly, Bouin & Flemming, prepared according to the formulations given by Baker (1945) and Pantin (1948). Helly's medium only was used for Coenurus cerebralis, and C. cellulosae had to be fixed in a mixture containing 15% Formol; 70% Ethanol; and 5% Acetic acid since these were the only fixative materials available at the Veterinary Laboratory in Livingstone.

Exposure to Zenker, Helly and Bouin was continued for 6 hours while for Carnoy and Flemming 2 hours were found to be sufficient. Materials fixed in the first two, which contain mercuric chloride, were washed in running tap-water overnight. Dehydration was carried out through graded Ethanols up to 96%, and the tissues were then cleared in a 50/50 (v/v) mixture of Methyl benzoate & Ethanol, before transference to pure Methyl benzoate. Exposure to this was continued overnight before the samples were transferred to Benzene for 2 hours in each of 2 changes. Embedding was completed in the third change of Paraffin Wax at 60°C (M.P. 56°C).

Sections were cut with a Rocking-microtome set at 6μ. For the examination of the cuticular elements Mallory's triple stain (Gurr) was used according to the method of Pantin (1948) while Heidenhain's Haematoxylin, or Ehrlichs' Haematoxylin counterstained with Eosin were used for the cellular elements.

Electron Microscopy: The cysts were cut into small pieces of approximately 2 mm. square and fixed for 1 hour in 1% osmium tetroxide buffered at pH7.5 with veronal-acetate (Palade 1952). After washing for 1 hour in veronal-acetate buffer the samples were dehydrated in graded ethanols up to absolute ethanol, one hour in each. Infiltration was carried out in a mixture 50/50 (v/v) of absolute ethanol and a mixture 73/7 (v/v) of N-butylmethacrylate and M-butylmethacrylate for 1 hour. This was followed by three changes of the N- and M-butylmethacrylate (93/7) without ethanol for successive 2 hour periods. The material was next transferred to a mixture of 93/7 N- and M-butylmethacrylate to which a catalyst, 1% (w/v) benzoyl peroxide, had been added. After 4 hours exposure the samples were transferred in the medium to a 00-gelatin capsule within which hardening was completed after 48 hours at 58 - 60°C. The blocks were prepared, trimmed and sectioned as required with a Huxley ultra-microtome set at 250 Å. The sections were examined and micrographs taken with a Siemen's Elmiskop I electron-microscope.

Histochemistry: Apart from the obvious limitations imposed on the processes of fixation, dehydration, embedding etc. by the chemical lability or solubility of certain of the chemical components, in tests designed for their histochemical recognition, there are problems of a

more recondite nature which influence the results of histochemistry. This remark applies particularly to the problem of fixation of the tissues. It is well recognised that for certain tests some fixative agents are more suitable than others and, although explanatory hypotheses have been advanced in some cases, the success or otherwise of a given procedure is largely an empirical fact. Its use, therefore, rests upon its general acceptance by workers with a wide experience in the field.

The methods adopted in the present work have been selected largely by reference to the recommendations of the authors responsible for their introduction and to the general opinion among histochemists as regards their validity and significance.

The following procedures and tests have been applied:-

A. General Tests

1. Basiphilia — Baker - Personal communication
2. Acidophilia — do.
3. Hime's triple stain — Hime's and Moriber (1956)

B. Carbohydrate Tests

4. Periodic Acid/Schiff Tests (P.A.S.)
   - (a) McManus method — McManus (1948)
   - (b) Intensified McManus method — Hale (1953)
   - (c) Hotchkiss method — Glick (1949)
   - (d) "After Hotchkiss" method — Pearse (1953)

5. P.A.S. after blocking and extraction (on P.A.S. positive materials)

   (a) Acetylation (acetic anhydride and pyridine) McManus (1960)

   (b) Acetylation/Deacetylation (KOH) McManus (1960)

   (c) Deamination ($NaNO_2$) Lillie (1954)

   (d) Pyridine extraction Pearse (1953)

6. Chromic Acid/Schiff test (Bauer's method) Pearse (1953)

7. Best's Carmine Pearse (1953)

8. Malt diastase digestion followed by P.A.S. or Best's Carmine McManus (1960)

9. Metachromasia (Toluidine blue) Pearse (1953) Baker (1960)

10. Metachromasia after blocking

    (a) Methylation (CH_3OH + HCl) McManus (1960)

    (b) Methylation/demethylation (KOH) do.

11. Alcian blue (8 GS)

    (a) Neutral Steedman (1950)

    (b) Acidic (3% acetic acid aqueous) McManus (1960)

12. Methylene blue extinction (Veronal-Acetate Buffers) Pearse (1953)

13. Sulphation by Sulphuryl Chloride or H_2SO_4 followed by Methylene blue (in Phosphate buffer at pH 2.4) Kramer and Windrum (1954)

C. Nucleic acid Tests

14. Feulgen for DNA — Feulgen and Rossenbeck (1924)
15. Pyronin/methyl green for RNA
16. Pyronin/methyl green after Ribonuclease (obtained from saliva) — Bradbury (1956)

D. Amino-acid Tests

17. Sakaguchi (for Guanidine derivatives) — Baker (1947)
18. Millon (for Phenolic groups) — Baker's modification (1956)
19. Xanthoproteic test (for Phenolic groups) — Lillie (1954) Baker (personal communication)

E. Lipid Tests

20. Sudanophilia
    (a) Sudan IV (Herxheimer's Medium) — Pearse (1953)
    (b) Sudan Black B — Baker (1956)
    (c) Sudan Acetylated — Casselman (1954) (personal communication)
21. Sudanophilia after extraction — Casselman (1959)
    (a) Hot pyridine
    (b) Cold acetone
22. Nile-blue sulphate (Acid and Neutral Lipids) — Cain (1947)
23. Acid haematein (for Phospholipids) — Baker (1946)
24. Acid haematein after extraction (hot pyridine) — Baker (1946)

25. Liebermann (for cholesterol) Lison (1953)

F. Calcium

26. Cobalt-chloride Casselman (1959)

27. do. after extraction with 2% Nitric acid Baker (personal communication)

The authorities quoted in the above list are not necessarily the authors of the particular tests, but are those whose instructions have been followed in the present work. In addition to the tests listed above trials were made with various other tests including Mayer's mucihaematein test (Lillie, 1954) and the Colloidal iron test of Hale (1946). While the first of these is a histological rather than a histochemical test, the second appeared to offer no additional advantage over the metachromosia and basiphilia tests for acid-mucopolysaccharides, and according to Pearse (1953) the specificity of this test both in its original (Hale 1946) and modified forms (Rinehart and Abu'l Haj, 1951) is doubtful. These tests were, therefore, not systematically applied and the results obtained with them are discounted in the present work.

Fixation. Although alcoholic fixatives are usually recommended for tissues that are to be subjected to carbohydrate tests, there appears to be no general agreement as to which of the various formulations is the best (Hale (1956) and Casselman (1959) have reviewed the subject). In the present work three fixatives were used, namely,

Carnoy, Alcoholic formalin, and Picro-alcoholic formalin, and the results are discussed and compared below. A non-alcoholic fixative, that of Zenker, has been recommended by Baker (1960). P.A.S. tests were therefore carried out, on all tissues to compare Zenker fixation with alcoholic fixation. Zenker fixative was also used, following the appropriate authorities' recommendations for material to be tested by Sakaguchi and the Xanthoproteic test (Nos. 17 and 19). For the mercuric nitrite test (Millon No.18) the fixative used was 4% Formaldehyde in 0.7% saline. Carnoy's fluid was used for the general tests 1-3 and for the Nucleic acid tests 14-16.

In fixing material for Lipid tests Baker's Formol-calcium medium was exclusively employed, following the general practice of other workers. In the special case of Phospholipids the post-chroming procedure of Baker (1946) was adopted (5% Pot. dichromate 1% calcium chloride).

The Casselman (1959) technique to test for calcium was applied to material fixed in Ethanol and acetone (1:1) according to the procedure outlined in the original description and also on material fixed in a mixture containing 4% Formaldehyde, 80% methanol and 2% pyridine (Baker - unpublished).

Embedding etc. The standard procedure of paraffin embedding as described above under histology was used for preparing all test materials excepting only where the Millon test (No.18) and the Lipid tests (20-25) were to be used.

Since the Millon test was to be carried out by

boiling the sections in the mercuric-nitrite reagent they have to be embedded in Collodion to prevent their disintegration under test. Sections were, therefore, cut from Collodion embedded tissues with a sliding microtome set at 30μ.

Tests for Lipids were carried out on material prepared as follows:- After fixation the tissues were washed and embedded in 25% gelatine in water to which was added 0.25% sodium p-hydroxybenzoate to prevent contamination with moulds and micro-organisms. Sections were cut with a freezing microtome set at 10-15 μ and, after staining, mounted in Farrants' medium.

A summary of all procedures is given in Appendix I and the formulation of reagents is summarised in Appendix II.

Part II: Section I:

General morphology:- In the majority of species of Taeniid tapeworm the larval form at the completion of its development in the intermediate host consists of a bladder or cyst composed of a generalised parenchymatous tissue surrounding a fluid-filled cavity. The whole is invested with a distinct cuticular covering. This cavity wall is invaginated at one point to form a scolex which after evagination in the definitive host will become the hold fast or head region of the adult tapeworm. The scolex is formed of more compact cells which have a more distinctly differentiated appearance. The scolex contains a central cavity which is continuous with the exterior (scolex canal) and is lined by cuticle which is continuous with that of the bladder surface. On the canal surface are developed suckers and in some species hooks which will become the attachment organs in adult life. This structure is shown diagrammatically in Figure/ 1(I to IV & VI). Among the species discussed here only Cysticercus bovis is lacking hooks. By common usage a cysticercus is a cyst with a single scolex and in the present work, the species C. bovis, C. cellulosae, C. tenuicollis, C. pisiformis and C. fasciolaris were always found in this condition. Nevertheless, Crusz (1948a) has described a process of endogenous budding in C. tenuicollis and has recorded a single case in this species of a cysticercus containing a daughter cyst. He has also described exogenous budding in C. pisiformis (1948b) but in his material the daughter

cysts which were derived by annular fission were acephalous.

Earlier authors, however, have described varying degrees of polycephaly in C. pisiformis (Schaaf 1905, Young, 1908); in C. fasciolaris (Lupke 1892, Dollfuss 1938, Clapham 1942, - Multiceps macracantha, Clapham 1942 = H. taeniaeformis,(Batsch 1786) according to Crusz 1948b - and Kuntz 1943) and in C. cellulosae, (Lent 1942).

The only polycephalus cyst studied in the present work was the single specimen of the true coenurus, in which polycephaly is the normal condition, namely Coenurus cerebralis (Fig. VI). This cyst was ovoid in shape measuring 20 x 10 x 10 mm. and containing 37 scolices at different stages of development. The heads were dispersed over the bladder wall in distinct groups, a feature which is characteristic for the species. This is obviously a comparatively small specimen of a species which can exceed 50 mm. in its major diameter (Morgans & Hawkins 1949) and may contain more than 700 scolices Clapham (1941). In older-standing cysts of this species there is sometimes an evagination of the scolices in situ in the intermediate host, a phenomenon which has long been known, Hall (1910). This was not observed in the present specimen. Evagination of the scolex on the other hand, is a constant feature of C. fasciolaris in the later stages of its development in the intermediate host. In specimens of 40 days plus scolex evagination occurs and a cell proliferation commences in the post-scolex or neck region and the larva proceeds to strobilate.

No observations were made on specimens exceeding 60 days in age, but by this time the larva has the appearance of a small adult tapeworm of about 12.0 mm. in length of which the anterior 7 mm. are taken up by a head and strobilated region of about 1 mm. in width. The body terminates in a bladder measuring 5 mm. in length by about 3 mm. in width (Fig.1(V)). By this stage up to 70 segments are recognisable in the strobilated region. This size can be exceeded by a large margin in older specimens, thus Rees (1951) refers to a specimen measuring 150 mm. in length and containing 544 segments. Nelson (1924) identified genital rudiments in segments 25 - 87 of a specimen containing 223 segments but this condition has not been recorded by any other observer.

The remaining species of cysticercus do not show such marked morphological characteristics as Coenurus cerebralis or Cysticercus fasciolaris, they do however show minor differences between themselves and are thus readily recognisable. C. bovis and C. cellulosae are both ellipsoidal with their major axes reaching 7 mm. and 10 mm. respectively in our material (although C. bovis can also reach 10 mm.). The head is situated such that it is bisected by the plane of the smallest circumference in both species. This is sometimes described, albeit inaccurately, as the equatorial plane. The head occupies a large proportion of the central vacuole. These species are morphologically distinguishable only on the basis of the presence of hooks in C. cellulosae and their absence in

C. bovis Figs. 1(I & II & 2).

In C. pisiformis and C. tenuicollis the bladder region is considerably enlarged and in them the head is confined to the anterior extremity which is separated from the bulk of the bladder cavity by a partially constricted "neck" region. This is less distinct in C. pisiformis than in C. tenuicollis in which its prominence gives the cyst its name (Fig. 1(III & IV). The greatest lengths of the present material were 10 mm. and 40 mm. respectively for C. pisiformis and C. tenuicollis. Both these species may attain double the lengths quoted, but while larger cysts were observed it was found more convenient to work with smaller ones.

Part II: Section II:

Histology of Mature Cysts:- It is convenient to record the histological findings under the two headings A. Cuticle, B. Parenchyma. Under each heading the mature cyst of C. bovis is first described in detail and the findings for C. tenuicollis, C. pisiformis, C. cellulosae and Coenurus cerebralis are then presented in comparative form. The description of the cuticle of the first three species follows closely the published account (Siddiqui in Press 1962). C. fasciolaris which was the only species for which material representing all stages of development was available is described separately in Section IV (p. 52).

A. Cuticle

Cysticercus bovis:- The thickness of the cuticle shows little variation between individuals and appears to be independent of the size of the cyst, although the range of size was from 4 to 7 mm. in major diameter. This variation in size probably represents differences in the degree of completed development since the suckers were more fully developed in the larger cysts than in the smaller ones. The cuticle varies markedly in thickness within the individual cyst. On the invaginated head it is 10 μ thick (Fig. 9) and from here it gradually diminishes down to 0.3 μ over the surface of the bladder (Figures 6 - 8). When sections are stained with Mallory's triple stain, three layers are readily distinguished in the cuticle; they are a blue staining external layer, a red staining middle layer

and a blue staining basal layer. When stained otherwise, for example with Heidenhain's or Ehrlich's haematoxylin the cuticle is unicolorous and the distinction between the three layers is lost. Electron micrographs from 2,000 - 80,000 diameters (Figs.10 - 12), however, fully confirm their existence.

The external layer of the cuticle is very thin, and is of the order 3×10^{-5} mm. (30 $\mu\mu$). In the invaginated cuticle of the head this layer is not evident although blue-stained fragments in the canal may represent its desquamated remains. (Unfortunately there was no opportunity to examine this region under the electron microscope). Elsewhere, over the whole cyst surface the external layer is continuously present. Beneath it is the middle layer which forms the bulk of the cuticle. This layer, which appears homogeneous under the optical microscope varies greatly in thickness in the different regions of the cyst, and it is variation in the thickness of this layer which produces the described variations in the thickness of the cuticle. The middle layer is bounded internally by the basal layer. This layer, which in Mallory-stained optical sections is very pronounced and much more readily recognizable than the external layer, is much less distinctly delimited than the external layer in electron-micrographs. Its thickness is variable but is of the same order as that of the external layer (Fig. 11) and it has a fibrous appearance.

The three cuticular layers show marked differences in their microstructure. While the external layer appears

to be homogeneous and the basal layer appears to be fibrous, it is not possible, on account of extreme thinness, to assert with any confidence their actual structure, even with magnifications as high as x 80,000. They do however show quite a different appearance from the middle layer. This latter appears to consist of a reticulated ground substance enclosing numerous sub-spherical vacuoles of varying size giving the whole an irregular honeycomb appearance (Fig.11 & 12). The vacuoles in places appear to be disposed in rows or lines sometimes traversing the thickness of the layer obliquely and sometimes lying parallel to the surface. In places where the ground substance between the vacuoles is less dense it seems to have a granular structure. In the innermost zone of the middle layer, occupying about one third of its total thickness, there is a series of dense discrete bodies of truncated sausage-shape, sometimes with a constriction near the mid-point in their length. These bodies reach a maximum size of about 0.15 x 0.05μ . Bodies with a similar appearance occur in the parenchyma. Read (1955) has a photomicrograph of the cuticle of an immature proglottis of Hymenolepis diminuta in which there are dense bodies in the same zone of the cuticle of which the size and shape is of the same order as in those described here. He suggests that these may be mitochondria. Threadgold (1962) carries the implication of Read's possibility further when he asserts firmly from his study of Dipylidium caninum that the whole cuticle is an extension of the cellular parenchyma. We shall

return to this point after discussing the parenchyma and the histochemical findings.

The surface of the cyst is densely covered by fine hairlike processes whose nature has been the subject of debate between earlier workers. They vary in length from about 0.2μ to 0.3μ and are longer in those regions where the middle layer of the cuticle is thicker (compare Figs. 6 & 8). On the invaginated head, however, where the middle layer of the cuticle reaches its maximum thickness, these hairs are entirely lacking (Fig. 9). With Mallory's stain the blue colour of the external cuticle is not evident on the hairs and they appear as weakly stained pink structures. They are also feebly stained when haematoxylin is used. The detailed structure of the hairs is therefore impossible to resolve with the optical microscope: Electron micrographs at lower magnification (Fig. 10 at x 2,000) show the hairs as outgrowths of the external layer, but at higher magnification (Fig. 12 at x 80,000) they prove to consist of two cuticular components. They have a core composed of a villus-like outgrowth of the middle layer of the cuticle which is widest at its base and tapers abruptly at its apex having an approximate mean diameter in the middle portion of $7 - 8 \times 10^{-5}$mm. This is invested with a continuation of the external layer whose thickness is uniform and equal to its thickness on the cyst surface. The basal layer of the cuticle appears to have no connection with the superficial hairs and is not represented in their structure.

Cysticercus tenuicollis:- The structure of the cuticle of this species is basically the same as that of C. bovis. It is of about the same thickness and shows the same order of variation in the different parts of the cyst. Figs. 15 - 18 illustrate these variations. The superficial hairs in this species are different in their appearance. The electron-micrograph (Fig. 19) shows them to be at least 0.5μ long which is perhaps twice as long as those of C. bovis, although it is difficult to determine the length of such hairs since the sections obtained probably all suffer from some degree of obliquity in their cutting. The diameter (0.02μ) of the superficial hairs is about equal to that of the hairs in C. bovis. They are, however, more sparsely distributed and are some 0.13μ apart whereas those of C. bovis are only distant by 0.05μ. Thus the hairs of C. bovis which appear to be less than half the length of those of C. tenuicollis are about 2½ times more numerous. Fig. 19 shows a number of electron dense discrete bodies which are more generally distributed throughout the middle layer of the cuticle and are not confined as in C. bovis (Fig. 11) to the interior third zone. They are not so elongate in shape but are more rounded in outline, and smaller (c. 0.02μ). There is evident in this section a local deep extension of the cuticle suggesting perhaps an extension into the cellular zone of the larva.

Cysticercus pisiformis:- Figures 20 - 23 show the variations encountered in the cuticle of this species. It is thickest

on the head as in the previous species (c.10μ) and thinnest on the bladder, diminishing to less than 1μ in this region. The superficial hairs are much more sparsely distributed and their distribution is discontinuous. They occur in groups which are irregularly scattered. On the bladder region the cuticle itself is thrown into fairly regular folds appearing in section as indented blocks separated by deep grooves (Figs. 21 & 22), whereas on the head it is quite smooth. This is in contrast to C. bovis whose bladder cuticle is relatively smooth, and to C. tenuicollis where the folds of the bladder cuticle appear to be quite irregular. In the electron micrograph (Fig. 24) we see a condition very similar to that found in C. bovis. In both these species the vacuoles (electron transparent areas) are more distinct and discrete than they are in Fig. 19 of C. tenuicollis. The mitochondria-like bodies are large in C. pisiformis as they are in C. bovis, and again they are more confined to the internal zone of the middle cuticle. The basement layer is much more irregular in Fig. 24 and appears to recede internally before evident deeper extensions of the cuticle recalling the one shown in C. tenuicollis (Fig. 19). Completely isolated from the cuticle there are discrete areas which appear to repeat the structure of the middle cuticle although they are not bounded by the innermost element of the cuticle. They represent perhaps the deepest penetration of the cuticle itself and serve to connect this with the cellular elements below. In the microstructure

of its surface this species also shows marked differences from the other two. In these the surface is flat and interrupted by the perpendicularly placed subcylindrical hairs while in C. pisiformis the surface is not flat but composed of a series of contiguous sub-hemispherical prominences a few only of which extend outwards to form hairs.

In studying the above three species fixation for optical microscopy employed four solutions for comparison. Good consistent results were obtained for Mallory staining and routine Haematoxylin - Eosin staining after fixation in Bouin, Helly, Zenker and Carnoy. There appears to be little to choose between these four but since Carnoy and Zenker are indispensable for histochemistry, further work on histology was confined to their use. Flemming was unsatisfactory since tissues fixed in it show poor chromophilia. Its use was abandoned.

Cysticercus cellulosae:- Sections of this species are so similar to those of C. bovis that they cannot be distinguished (apart from the presence of head hooks in C. cellulosae).

Coenurus cerebralis:- The cuticle of the bladder has a smooth surface and is thin as in the cysticercus species (c.2μ) the surface is sparsely haired resembling C. tenuicollis. This species has an added interest in that it contains numerous scolices at varying stages of development. The head cuticle is much thicker than the bladder cuticle but it varies somewhat according to the stage of development of the scolices. In those scolices which have not yet

developed suckers the cuticle may be as thin as 3.5μ . When the suckers and hooks are well developed the cuticle thickness has increased to 10μ .

B. Subcuticular Elements or Parenchyma

Below the cuticle the cysticercus contains both cellular and fibrous elements. In the head region two parts are well-defined, a deeper medullary zone separated by muscle fibres from an outer subcuticular zone or cortex. In the bladder region, on the other hand, the zonation is less distinct and there is clear continuity between medullary and cortical elements, which merge into each other.

Head cortex has a characteristic appearance, in which the nuclei are elongate and concentrated at one level forming a kind of subcuticular palisade (Figs. 9, 13 & 14). The long axes of the nuclei are perpendicular to the surface (Figs. 9 & 13) and the nuclei are sometimes very crowded. The cell boundaries are difficult to define, but in some sections (Figs. 10,13&14)the cytoplasm around the palisade nuclei is thin at the level of the nucleus, but it extends as a narrow band both internally and externally to the nucleus to give the appearance of a spindle shaped cell. Externally the cytoplasmic extensions appear to branch and extend up to and even into the cuticle (Figs. 9 & 13). Frequently there is a tendency for the cuticle to separate from these cytoplasmic extensions. This separation has often a quite regular appearance and it is impossible to

decide whether it is a genuine structural feature or whether it is an artifact. Figure 18 shows cuticle which is separated in one part, and is firmly in continuity with the subcuticular cytoplasm in another.

We have failed to obtain any preparations for either photo - or electron microscope which present so definite a picture of the relationship between cuticle and cortical cells as that given by Threadgold (1962). From our preparations it is doubtful whether Threadgold's interpretation could have been reached independently, but on the other hand they contain no evidence which could be taken as contrary to Threadgold's views which, in the main, they appear to confirm.

A similar appearance is found in the bladder cortex, but here the nuclei are not so far withdrawn from the cuticle and the palisade effect is much less regular (Fig. 14).

The medulla in both head (Figs. 9 & 13) and bladder regions (Fig. 14) is composed of sparsely scattered nuclei surrounded by exceedingly sparse cytoplasm which continues as fine strands forming a fine mesh supported by fibrous elements. The whole appears to be an entirely undifferentiated syncytium. There is no differentiation of the cellular elements around the central body cavity, and no evidence at all of a limiting membrane.

The calcareous concretions which have been emphasised by many workers were abundantly found in cortex and medulla of the head and neck regions, but are rare in the bladder region.

Muscle fibres occur in defined groups forming a radial and longitudinal series, between the palisade nuclei of the cortex and the cuticle. The subcuticular cell processes traverse the muscle bands to reach the cuticle (Figs. 6,7,17 & 22). The difficulty of orientating sections with respect to the site of the bladder from which they are taken makes it difficult to determine the direction of the long axis of the various fibres. On the head, however, orientation is more easily achieved and here it is evident that the outermost muscles are circular while the inner are longitudinal. The radial muscle fibres are particularly numerous and well developed in the suckers of the proscolex. Scattered muscle fibres are found in the medullary region but are more rarely grouped in bundles in this zone.

No use was made of special histological staining techniques to identify structures such as myoblasts, neurones and neurofibres although such elements have been described by earlier workers (Young, 1908 and Voge 1962). The tinctorial results of some of the histochemical procedures which are described in Part II, Section III, revealed histological information in addition to that described above which was derived from routine Mallory or Haematoxylin/Eosin staining. In particular, Baker's acid haematein test for phospholipids produced preparations in which muscle fibres were very clearly delineated - presumably from staining of their sarcolemma - and in which the basement membranes lining the excretory channels in the medulla were

sharply defined. Acid haematein also stains mitochondria differentially, and these bodies were readily recognisable in the subcuticular region especially concentrated in the subcuticular cytoplasmic strands immediately adjacent to the cuticle (Figs. 43 & 45).

The basic dyes such as toluidine blue and methylene blue reveal differences in form between the nuclei of different regions (Figs. 29 & 30). The Feulgen test for DNA shows up differences in the dispersion of chromatin material. In the subcuticular region the nuclei varied from types in which the chromatin was dispersed in the form of small discrete spots to those in which it occurred as a single large aggregate either spherical or horse-shoe shaped in form.

Part II: Section III:

Histochemical Studies:- The results obtained from histochemical procedures are summarised in Table I, from which it can be seen that there is little evidence of major differences in chemical structure between the different tapeworm species. The results are, therefore, described collectively for all the species studied, excepting C. fasciolaris which is treated separately in Section IV. The dominant concern throughout this section is with the cuticular integument and its probable chemical nature, and reference to the subcuticular matrix and cellular elements is restricted to the part they play as controls for comparison with the cuticle in the province of histochemical technique, and to the separate question of their role in food storage.

The cuticle, in both its major facies, namely the thin investing bladder cuticle and the thick invaginated head cuticle shows a strong affinity for both eosin and safranine, and is thus revealed as a complex containing both basiphilic and acidophilic components. When Himes & Moriber's triple staining process is applied the cuticle appears a deep yellow colour indicating the probable presence of protein from the combination of the naphthol-yellow S with the available amino groups, and the non-nuclear parts of the sub-cuticular tissues stain red indicating polysaccharide materials in the cytoplasm and fibrous elements (Fig. 25). The nuclei have a bluish-green tinge, presumably from the combination of the colours of

naphthol-yellow S attached to protein and the Azure A attached to D.N.A.

Carbohydrates:- The apparent absence in the cuticle of Schiff-reacting components in the Himes & Moriber test is remarkable and the material was, therefore, subjected to a more detailed analysis using the various recommended procedures of histochemical application of the Schiff reaction. Although three distinct periodic acid/Schiff procedures were applied on material fixed in four different fixatives, making 12 tests in all, there was a consistent failure (Fig. 24) to reveal the presence of periodic acid-oxydisable materials in the cuticle of bladder and head of all species with one sole exception, namely that of the immature scolices of Coenurus cerebralis. We shall return to this exception at a later point in the thesis. As might be expected from its lower sensitivity (Casselman 1959), Bauer's test, in which the oxidant is chromic in place of periodic-acid, also failed consistently to reveal Schiff positive substances in "mature" cuticle. A.J. Hale's (1953) method of pretreatment with weak alkali, which has been shown to intensify the PA/S reaction of certain weakly reacting mucoid substances, had no visible effect on cysticercus cuticle which remained obstinately PA/S negative. In all these cases the subcuticular tissues provided a good control for assessing the technique since PA/S positive materials were abundantly identified in them, and glycogen granules (vide infra) were particularly evident (Figs. 26, 31, & 33). Since the technique has been properly

applied we must conclude that the cuticle is either devoid of carbohydrate materials, or that those that are present are such that they lack oxydisable OH groups. The second of these provides the more promising working hypothesis and its adoption is supported by the evidence from the cuticle of Coenurus cerebralis in which we find in one and the same cyst, PA/S negative cuticle on the bladder and mature scolices, and cuticle giving a strong positive PA/S reaction on the immature scolices. Unfortunately, it was impossible to carry out differential tests on this Helly-fixed material which was the only material available of this species, nevertheless we shall produce evidence from studies on developing C. fasciolaris in Section IV that the positive PA/S reaction in the cuticle of tapeworm cysts is due to OH groups in carbohydrate materials and not to lipids or $CHNH_2$ groups in other substances.

A number of carbohydrate materials is known which do not produce reactive aldehyde groups on exposure to oxidants and, therefore, fail to combine with Schiff's reagent to produce the detectable colour complexes. Such substances are found particularly among the complex acid mucopolysaccharides. In some cases the negative PA/S reaction is chemically explicable on the grounds of absence of the necessary adjacent hydroxyl (1,2 glycol) or 1,2 hydroxyl amino groups whose oxidation product after cleavage of the carbon-carbon bond is an aldehyde. Such an example is fully sulphated heparin (Jorpes et al, 1948)

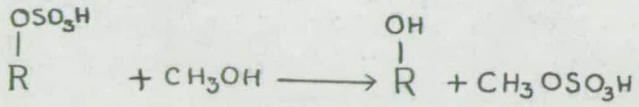
in which the presence of 3 sulphate groups per monosaccharide unit blocks the aldehyde yielding groups. Most known examples of PA/S negative polysaccharides cannot be so simply explained, however, and it appears that more complex mechanisms are involved such as polymerization or chemical association or union with protein. When combination of protein with polysaccharide causes inhibition of its PA/S reaction, breakdown of the mucoprotein by alkali (as in Hale's intensification procedure) might be expected to release its PA/S reactivity. This did not occur, however, in the present examples. At the tissue level there is even greater difficulty in the interpretation of results but examples can be quoted in:- the chitin of insects - a neutral mucopolysaccharide - which is PA/S positive when "pure", but after sclerotisation by the tanning of interdispersed protein it becomes PA/S negative (Richards, 1952); the chitin of the snail radula (Runham, 1961) which similarly is PA/S positive on secretion but upon sclerotisation becomes PA/S negative; or in the mucin of snails which contains the complex acid mucopolysaccharide limacoitin the PA/S reactivity varies according to A.J. Hale (1957) in relation to species and site of production. It may, therefore, be stated at this point that failure to identify reacting aldehydes does not rule out the possible presence of carbohydrate which could occur in PA/S negative tissues either as neutral mucopolysaccharide in association with protein or as complex acid mucopolysaccharide.

Various staining techniques were now applied and positive staining of both types of cuticle was obtained with Mayer's mucihaematin and Hale's colloidal iron methods. These methods, however, which were introduced for the identification of mucoid substances are non-specific and McManus & Mowry (1960) have even described the first as of historical interest only. They do, however, indicate the presence of basiphilic components, and this basiphilia was, therefore, explored further. The cuticle has a very strong affinity for alcian blue in both acidic (pH 2.6) and neutral media and sections stained with this dye show the cuticle much more deeply stained than any other part of the cyst (Fig. 27). The presence of such markedly basiphilic materials strongly implies the presence of acid mucopolysaccharides. The methylene blue extinction technique offers a means of quantitative assessment of this basiphilia. The method of Pearse was applied to determine visually the pH of the end point where the methylene blue binding capacity of the cuticle is lost. Figure 28 shows the strong affinity for methylene blue at pH 4.1 but this is lost below pH 2.2 (Fig. 29). Toluidine blue was used to test the material for metachromasia. The result is shown in Figure 30 in which the nuclei appear orthochromatically stained a bright blue colour while the cuticle and the cytoplasmic portion of the subcuticle are distinctly metachromatic in appearance. In this, as in the other colour prints of stained sections, the colour values

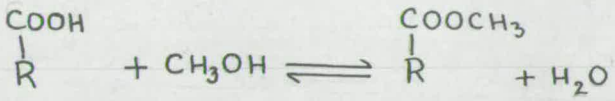
are inaccurate and while there is clear evidence of metachromasy it does not show the same degree of redness that direct observation detects. In other words the metachromasia in the cuticle is a distinct γ metachromasia. Some differences were noted in the metachromasia of the different cuticle samples studied. Thus, dehydration in 70% ethanol rendered head cuticle orthochromatic much more readily than bladder cuticle in which the metachromasia was much more resistant. Also the metachromasia of C. bovis cuticle was more resistant to ethanol and water treatment than was that of cuticles of the other species studied. From these findings it would appear that cysticercus cuticle in its different parts can exhibit both the "true" and "false" metachromasia phenomena of Sylven (Pearse 1952). The significance of ethanolic differentiation is, however, not properly understood and has been the subject of differing opinions among workers. Pearse's comment that differentiation has no place in histochemical technique suggests that he would accept all the present findings as examples of metachromasia. Schubert & Hamerman (1956) have reviewed the subject of metachromasia and conclude that it occurs in the staining of high molecular weight substances which have free anionic groups. Such substances include acid mucopolysaccharides, nucleic acids, and certain acid lipids which polymerise to give high molecular weight micelles. Of these possibilities the failure to stain with methylene

blue below pH 2.2 tends to eliminate the nucleic acids, since nuclei do so stain. The Feulgen reaction reveals no concentration of D.N.A. in the cuticle and treatment with ribonuclease - whose activity was assessed by reference to the subcuticular cytoplasm - failed to reduce the intensity of pyronin/methyl green staining and hence it is concluded that R.N.A. does not occur in significant quantity in the cuticle either. Pearse 1952 mentions that highly polymerized carbohydrates or metaphosphates have also been shown to produce metachromasia but this is usually of β — type, and that β — metachromasia may be taken as indicative of carboxyl or phosphoryl groups. Acid mucopolysaccharides containing ester sulphate groups produce the most intense staining effects and it must be concluded that the evidence advanced so far in the present work points more strongly to substances of this type than it does to the other possibilities. Methylation with methanol and H Cl has been shown by various workers to esterify carboxylic acids, and to de-sulphate sulphate esters (McManus & Mowry 1959) thus eliminating the reactions dependent upon their presence. After methylation the cysticercus cuticle failed to stain with alcian blue and toluidine blue. Metachromasia was abolished after only 2 hrs. exposure at room temperature to methanol whereas complete elimination of basiphilia (alcian blue) required 6 hrs. at 60°C or 48 hrs. at room temperature. Demethylation of methylated materials clearly cannot restore

a basiphilia or metachromasia due to sulphated esters since the loss of the SO_3H group is irreversible, as follows:-



whereas the methyl ester resulting from a carboxyl group can be saponified by alkali thus:-



Saponification restored a metachromasia with toluidine blue indicating the second type of reaction: this was never so intense nor so markedly red, however, as in the original cuticle before methylation. Its near-violet tinge suggests a β-metachromasia. The loss of the original γ-metachromasia indicates its probable association with sulphate ester groups in an acid mucopolysaccharide. Further evidence of an irreversible change following methylation is found in an end point change for methylene blue staining. The original end point of pH 2.2 is raised to pH 4.1 in cuticles saponified after methylation whereas the methylated cuticle has an end point approaching neutrality. In the same sections methylation does not destroy the affinity of nuclei

for methylene blue even at pH 2.2.

The conclusion from these tests is that the cysticercus cuticle in mature examples contains carbohydrate material in the form of complex acid mucopolysaccharides with ester-sulphate groups and carboxyl groups. The masking effects of these on histochemical tests prevents a determination regarding the presence or absence of phosphoryl groups in the chromatophore.

Proteins:- The yellow coloration of cuticle with Himes & Moriber's stain (Fig. 25) already indicates the presence of amino groups. Further tests were now conducted in an attempt to identify the amino-acids present. Unfortunately such tests are very limited at histochemical levels. Baker's (1956) modification of the Millon reaction was carried out on collodion-embedded sections. This reaction is given by proteins containing tyrosine (hydroxy-phenyl groups). A distinct rose-pink colour was produced in the protoplasm of the parenchyma and this contrasted strongly with the exceedingly feeble coloration of the cuticle. From this finding it must follow that if the cuticle contains tyrosine at all it is in small quantity.

The xanthoproteic reaction was very destructive of the tissues but did leave recognisable cuticle. This was quite devoid of any yellow or orange coloration which implies the absence of all aromatic amino-acids (tyrosine, tryptophane, and phenylalanine) and suggests that the doubtful Millon reaction should be regarded as negative.

The most reliable histochemical test which can be used in this sphere is Sakaguchi's test for guanidine derivatives. The Baker (1944) histochemical application of Sakaguchi's first test (employing ∝-naphthol) was used. A strong red colour in the cuticle contrasting strongly with the less heavily stained parenchyma indicated the probable presence of arginine in basic protein. This would account for the cosinophilia found in the preliminary testing.

Tests for the SH and SS groups of cystine and cysteine were not carried out.

In conclusion, we can infer the presence of basic protein, but have no information on its amino-acid components apart from the probable occurrence of arginine.

Lipoids:- In sections cut frozen from material fixed in Baker's formol-calcium medium a strong sudanophilia was observed in the cuticle of both head and bladder of all the species studied (Figs.37 & 40). Elsewhere in the sections there was a diffuse sudanophilia in the cortical cytoplasm and a strong sudanophilia in discrete droplets in the medulla. Paraffin sections treated with sudan colorants showed a similar distribution of sudanophilic components except in the medulla where the droplets were entirely lacking. The importance of fixation is shown by the results in Figures 41 & 42 where some degree of dispersion of the lipoids, identified by their sudanophilia, is associated with excessive exposure to formol-calcium fixative. In these examples the material was accidentally

left for 24 hours in fixative instead of the accepted 6 hours which latter produced the results shown in Figures 37-40. There appears to be no significant difference in the findings with different sudan colorants including Sudan IV, Sudan Black B, and Acetylated Sudan although their lipoid specificity is stated to increase in the order given. Cain (1950) regards Sudan Black B as having perfect specificity but Casselman (1959) has pointed out that oil soluble colorants can act as weakly basic dyes and produce non-specific staining of some tissue elements. This possibility was ruled out by the complete removal of Sudan colorant from all elements by 70% ethanol, and by the fact that decoloured sections were readily recoloured by re-exposure to the colorant. Sections extracted in cold acetone were different from non-extracted ones only in the absence of the medullary sudanophilic globules. In contrast to this Bouin-fixed material extracted with hot pyridine (Baker, 1946) was completely devoid of all sudanophilic components. We may conclude from these tests that all the sudanophilic elements in the cysticercus are lipoid or lipoid-containing. The medullary fat droplets, however, differ from the diffused lipoid of the cortical cytoplasm and from the abundant lipoids of the cuticle, in their facile removal by cold acetone and by paraffin embedding whereas the others are stable to these solvents and removed only by hot pyridine.

The ready solution of Sudan colorants in these tissues implies the liquid state of their lipoids at room temperature and the probability that they are to some degree unsaturated.

Oxidation by performic acid followed by treatment with Schiffs reagent produced a faint pink coloration of the cuticle only. Elsewhere there was no evidence of colour. A weakly positive PFA/S reaction in the cuticle may be interpreted as indicating the presence of unsaturated lipoids in which ethylene bonds are probably exidised to aldehydes by the performic acid. Bromine saturates such bonds, and accordingly bromination destroyed all traces of a positive reaction when the PFA/S reaction was subsequently applied.

When Nile-blue sulphate staining at 60°C was applied according to the method of Cain (1947) there was no obvious difference between sections stained with a 1% solution only, and others stained by 1% followed by a 0.02% solution. Moreover, there was no great difference between the results in frozen gelatine sections, and ordinary paraffin ones. A deep blue staining was produced in cuticle, cortical cytoplasm, nuclei and in the dispersed globules in the medulla. There were sometimes variations in the blue tone particularly in the medullary droplets which sometimes showed a slight magenta tone. Nevertheless, there was no distinct red tone in any part of the sudanophilic areas. The colour in the sudanophilic areas was removed by 70% ethanol in about 3 minutes at room temperature but the blue colour in the nuclei was very resistant to ethanol and was only removed after prolonged exposure. In sections stained by 0.02% nile-blue sulphate only, deep blue staining was obtained in all the parts stained by the other methods. In these sections ethanol treatment

removed the colour more readily than when 1% solutions had been employed. Cain (1947) has put this test into proper perspective and has exposed the error in the earlier views of Lison (1936) and others that a blue colour with nile-blue staining has no lipoid significance. Cain showed that a red colour was produced in 'neutral lipoids' whereas fatty acids and acid lipids (phospholipids) stained blue. His interpretation that a red colour is due to solution of the oxidised nile-blue, or red oxazone and the blue colour due to soap formation by combination of the free base with fatty acids or acid fats, the free base or oxazine being present in significant amount only in very dilute solutions of the dye. The data obtained in the present sections appear to accord more closely with Lillie's (1956) interpretation/is that two blue phenomena occur, one a simple solution in the lipid with a blue colour in acid fat due to an indicator property of the dye, and the other a blue salt or soap formation such as Cain deduced. In cysticercus a deep blue was produced in both 1% and 0.02% dye solutions, yet the oxazine could only be presumed to occur in the weaker solution. Our findings are not completely in accordance with expectations from Lillie's data in so far as there was no difference in shade between the blue colour which was readily extracted by ethanol, and that which resisted ethanol extraction. This latter was in Lillie's experiments a paler greener shade. One cannot place much emphasis on slight differences in shade

as between the results of different workers, since it is well known that different samples of the dye have different tinctorial qualities. Lillie (1956) found considerable variation between eight separate samples used in his work. The sample used in the present work was prepared by B.D.H. and kindly supplied by the Cytological Laboratory of Oxford University where its value for distinguishing between 'neutral' and acid lipoids had been confirmed.

Whatever might be the complete explanation of the findings the conclusion is in accord with both Cain's and Lillie's general conclusion that a blue colour in the sudanophilic areas is a sign of acidity whether this be due to free fatty acids in solution in the lipids or to acid lipoids such as the phospholipids.

It is generally agreed that Baker's (1946) acid-haematein test is specific for phospholipids when it gives a positive reaction in sudanophilic elements. A control can be employed by simultaneous processing of hot-pyridine extracted sections. Occasionally hot-pyridine extraction was unsuccessful, and instead of removing all lipids it merely served to translocate them. An unsuccessful example is shown in Figure 46 where the cuticle has had its lipoids removed to the subcuticula whereas Figure 44 illustrates a successful example which compares strikingly with the unextracted section (Fig. 43). Cain (1947) has emphasised that the specificity of staining in this test is dependent upon correct timing of the procedures. The stain combines

with a chrome mordant for which the phospholipids have a relatively great affinity. Thus excessive chroming leads to fixation of chromium by other components, while insufficient differentiation with ferricyanide leaves the non-specific light blues and browns as false indicators. In the present work the application of the acid haematein technique was supervised by Drs. Baker and Cain whose authority thus confirms the results and their interpretation.

All cysticercus cuticles stained a dark dirty blue colour (Figs. 43 & 45) and a similar colour was observed in the sarcolemma of the muscles and in small discrete granular bodies in the cortical cytoplasm (Figs. 43 &45).These last are presumed to be mitochondria. The cuticle appeared uniformly stained and from this it is concluded that the acid lipoid dispersed throughout the cuticle substance (Sudan & Nile-blue) contains phospholipids, and it is to these that the acid nature of the cuticular lipoids is probably due. There was no evidence of a positive acid-haematein reaction in the fatty droplets in the medulla. They do not, therefore, contain phospholipids and are probably, in the main, simple stored fats of neutral type. Their blue reaction with nile-blue sulphate must be attributed to the presence in them of free fatty acids resulting from hydrolysis. The variation in tone could thus be interpreted as due to variations in their fatty-acid content.

Liebermann's cholesterol test was entirely negative in cuticular material, and it is concluded that there is no

significant steroid component in the cysticercus cuticle.

The strong Sakaguchi reaction indicating the probable presence of arginine, and the negative results obtained with Millon's and the Xanthoproteic tests suggests the possibility that the proteins of the cuticle have undergone some degree of tanning. The argentaffine test of Fontana was applied to test for the possible presence of reducing agents such as the polyphenols. The results were negative, but although they rule out the presence of polyphenols at the time of test, it does not follow that tanning of the cuticular proteins has not previously occurred. This remains an open question.

A metal substitution technique was applied to test the presence of calcium salts. Cobalt substitution was employed and calcium salts were detected only in the calcareous corpuscles (Fig. 47) which have been described above as being concentrated principally in the proscolex. Since cobalt does not replace calcium where this occurs as oxalate it is presumed that the calcium deposits in the corpuscles must occur as phosphate and/or carbonate. No calcium salts were detected in the cuticle.

Stored-food reserves:- Reference has been made to the lipoid droplets occurring in the medullary region. These, which are interpreted as probably triglycerides containing dissolved fatty acids derived from hydrolysis, are presumed to represent food reserves. They are especially abundant in the circumvacuolar region of the medulla but are much sparser in the peripheral medulla. They are rare in the head region. The principal food reserve material,

however, is glycogen which was identified by PA/S staining methods (Figs. 31 & 33) and by Best's carmine staining compared with enzyme digested controls (Figs. 32 & 34). (The cuticle stains deeply with Best's carmine, although it is PA/S negative. Malt-diastase had no effect on its staining reaction, however, and its affinity for Best's carmine is simply a reflection of its basiphilia). Glycogen occurs abundantly in the form of granular deposits in the circumvacuolar medulla, and less abundantly in the cortex and muscles. Glycogen is generally dispersed throughout the proscolex, especially in the medulla but it never appears in the great concentrations such as are found surrounding the bladder vacuole. It was not possible to determine the precise location of this glycogen which may be intracellular or may occur in the intercellular spaces.

The morphology of glycogen deposits in Platyhelminthes and other organisms has been long known to vary greatly. In comparing the differences in appearance of glycogen in nematodes and an acanthocephalan, von Brand (1939, 1940) suggests that they might have been due to differences in concentration. It seems a somewhat profitless exercise here, however, to place much emphasis on the form glycogen assumes in fixed prepared sections since such marked differences are produced by different methods of fixation. Following fixation in Carnoy and alcoholic formalin large glycogen granules of varying shape and size are observed (Figs. 31 & 33). When Zenker fixation is used the glycogen tends to aggregate in masses (Fig. 35) picro-alcoholic

formalin, in contrast to this, fixed the glycogen in the form of very fine particles along the parenchyma fibres and in the spaces of the reticular network.

Part II: Section IV:

Cysticercus fasciolaris: Studies on the developing larva.

Larvae were examined at fixed intervals of 10, 20, 40 and 60 days after infestation of the mouse-host by oral administration of embryonated eggs. At 10 days they are already cyst-like organisms containing a fluid-filled vacuole which comprises the major part of their volume. They appear as spheroidal bodies ranging from 0.4 to 0.6 mm. in diameter, or they may be ovoid and markedly elongate (Figs. 48 & 50). Their individual size depends to some extent, as Crusz has pointed out, on the density of infestation of the mouse-liver, size being inversely related to density. In this, as in the later stages of development material was larger and more advanced than the cysts described by Crusz for comparable ages of larvae. At this early stage there is already a distinct host reaction, the liver cells being strongly infiltrated in the pericystic zone by macrophages (Figs. 48 & 49). These are so closely adherent to the cyst itself that it is quite impossible to extract it cleanly from the host tissue. This fact, together with the relative difficulty of locating such small objects in the liver tissue, prevented their extraction and isolation: they were accordingly studied in sections of infested liver. Sections reveal an extremely simple generalised structure in which the

living tissues are disposed to form an enclosing wall around the central vacuole, and the whole is invested by a cuticle (Figs. 48 & 50). There is no organic differentiation of the living wall, whose appearance is the same everywhere, namely, that of a cytoplasmic mass enclosing numerous nuclei. The only variations noted were that the wall itself is of variable thickness, ranging from 15μ - 75μ , and at places there are nucleate cytoplasmic processes projecting into the vacuole (Figs. 48 & 50). Some living material appears isolated as islands in the vacuole. These may or may not be isolated, but this point was not explored by systematic serial sectioning. There is no evidence of clear cellular limits and the undifferentiated tissue is presumed to be syncytial. Moreover, at this stage differentiation has not proceeded far enough to indicate future cortical and medullary zones. Muscle fibres are not yet differentiated. Crusz identified myoblasts in his sections: they were not evident from the general histological techniques employed in the present studies.

The cuticle is clearly recognisable as a uniformly staining structure with haematoxylin stains around 1μ in thickness. Mallory's stain gives some indication of its probable multiple structure, but this cyst is strikingly different from the mature cysts of other species already described in that the blue staining basement layer is interrupted and does not form the

continuous limitation such as we found in mature cysts, and the outer blue staining portion is not identifiable. This last difference could be due to the fact that the host macrophages, which themselves are predominantly blue when stained with Mallory, obscure the outer layer. Alternatively, the outer layer itself may still be undifferentiated. These young cysts are fairly difficult to prepare in section and never produced the elegant microscopic picture that can be obtained with older material. The cuticle shows a marked tendency either to separate in toto from the underlying tissues, or it separates concentrically into inner and outer layers, the division occurring in the substance of the middle cuticle (Figs. 50 & 51).

Electron-micrographs (Figs. 52 & 53) show the cuticle as a honeycomb meshwork of moderately electron-dense material enclosing numerous vacuoles either containing less dense material or nearly devoid of contents. The transparent vacuoles which are more numerous in the basal than in the peripheral cuticle tend to be linearly disposed, thus forming channels or ducts which are directed either along the cuticle parallel to its surface, or across the cuticle perpendicular to the surface. Among the latter group there are some which are in direct continuity with the underlying tissues (Figs. 52 & 53). The hair-like superficial processes which were found in all the other

species are not recognisable in Figures 52 or 53 as such. There are cuticular processes, however, of a more irregular form, some of which are vacuolated, which are in intimate contact with the surrounding host tissues. This contact is so close that it is quite impossible in places to decide which is parasite substance and which is host substance. As with Mallory-stained optical sections the external cuticle cannot be identified with certainty from the electron-micrographs. There are places where the cuticular surface appears to be denser, but for the most part the confusion of host and parasite structures at the contact margin obliterates the definition of limits and the electron microscope adds little more on this point to the information obtained with the photomicroscope.

The parasite tissue has the same general appearance in electron-micrographs as that shown by the cuticle and it seems that these figures permit no interpretation other than that the cuticle is a modified peripheral condensation of the syncytial cytoplasm.

Sections of 10-day old cysts were subjected to a complete histochemical analysis and again the principal concern was with the cuticle. The results are summarised in Table II.

General tests showed through an evident eosinophilia and safraninophilia the presence in the cuticle of both acidic and basic components. Himes & Moriber's test produced an orange coloration of the

cuticle indicating PA/S positive substances in addition to protein. With the three histochemical PA/S tests 10-day old cuticle gave a positive reaction (Fig. 54). The positive reaction remained in the cuticle of sections tested after

(1) Diastase digestion

(2) Extraction of lipoids with hot pyridine, and

(3) Deamination with nitrous acid.

These tests exclude protein, lipoids and glycogen as the PA/S reactive substances which must, therefore, be mucopolysaccharides.

This PA/S reaction was blocked by acetylation and restored by saponification of acetylated sections in weak (0.2N) caustic soda. It was earlier thought that O-acetylation was readily saponified by weak alkali whereas N-acetylation was not (which is probably true) hence a reversible blocking by acetyl groups would indicate adjacent OH groups whereas irreversible blocking should occur when amino sugars were acetylated (McManus & Cason 1950). A.J. Hale (1953 & 1957) has shown that even though the amino group remain acetylated, provided the adjacent OH group be restored by saponification the substance will oxidise with periodic acid. Thus acetylation cannot distinguish between 1.2 glycols and 1 - amino, 2 - hydroxy groups. To determine whether these were neutral or acid in reaction the following evidence was obtained:

(1) Alcian blue positive staining in neutral and acid media, although much less intensively than in the other species studied

(2) Methylene blue stained the cuticle down to pH 4, but no staining occurred when the medium was more acid than this.

These findings suggest the presence of acid-mucopolysaccharides.

Toluidine blue failed to stain this cuticle. Hence, we conclude that although this cuticle contains acid-mucopolysaccharides they are significantly less basiphilic than the "mature" cuticles of Section III and their basiphilia is probably due entirely to carboxylic groups, i.e., they are simple and not complex acid-mucopolysaccharides.

Among the amino-acid tests Sakaguchi and the Xanthoproteic reaction were both positive. This latter test (for phenolic compounds) which was negative with Section II cuticles indicates a greater range of amino-acids in the young cuticle. (Unfortunately, the Millon test could not be carried out on this material since a sliding microtome for cutting collodion sections was not available in Edinburgh, and this material was not to hand when the other cysts were cut in Oxford).

This cuticle showed no differences from the other cuticles as regards lipoids. It was sudanophilic

(Figs. 56 & 57), stained blue with nile-blue sulphate and the positive stain with acid haematein (Fig. 58) was absent from pyridine-extracted sections (Fig. 59).

Reserve food materials were identified in the circumvacuolar tissues in small quantity in the form of fats (Figs. 56 & 57) and glycogen (Fig. 55).

It is concluded that the cuticle of the 10-day old cyst of C. fasciolaris contains basic and acidic protein, simple acid mucopolysaccharides and phospholipids.

At 20 days the main changes noted were as follows:-

(1) The infiltration of the adjacent liver tissue with macrophages and lymphocytes was considerably extended (Figs. 60 & 62) and closely applied to the cyst there were fibroblasts which had now replaced the macrophages as the principal investing host-elements. These cause a degree of contraction away from the liver tissue so that cysts with a developing host capsule can easily be removed from the liver at this stage (Fig. 61).

(2) The proscolex shows a relatively advanced stage of development (Figs. 60 & 61) - at this stage Crusz (1948c) could only recognise a proscolex anlage.

(3) The bladder wall is much thinner than at 10 days, and still does not show a distinction between cortex and medulla.

(4) Differentiated muscle fibres occur in the head region.

No electron-micrographs were taken of this stage, but optically there is no evident change in the cuticle although at this stage cuticular separation or splitting were never observed. Head cuticle is present now and since it is free of host cells its surface can be readily seen. Mallory stain does not reveal a superficial blue layer and in this instance we must conclude that it is absent. It should be recalled, however, that the blue external layer is also lacking in head cuticle of C. bovis etc.

Histochemically the cuticle at 20 days is unchanged from that of 10 days.

At 40 days the cyst is enclosed in a well-developed fibrous capsule of host origin (Figs. 63 & 64). The cyst cuticle is still inseparably attached to the host tissues so that it is impossible to excapsulate the cyst although the encapsulated cyst is readily removable from the liver. At this stage the cyst possesses a fully developed proscolex with a well-developed musculature (Figs. 65 & 66). Muscle fibres are still not evident in the bladder wall. There are no other significant changes.

The cuticle is histochemically unchanged.

At 60 days the picture is entirely different. The proscolex is now evaginated: this has been shown to occur soon after 40 days (Bartels 1902, Hutchison 1958). According to Hutchison 60 days is the earliest age at which this larva becomes infective to the definitive host. The larva now consists of three distinct portions, namely, head, strobilated region and bladder (Figs. 1(v) and 4 & 5).

The bladder tissues by this stage are quite clearly demarcated into a cortical and medullary zone. These zones are histologically comparable to the same zones in the mature cysts of C. bovis etc. (Figs. 69, 71 & 72). The medulla is composed of a syncytial network in which the nuclei are sparsely dispersed while the cortex has a palisade appearance and contains muscle fibres running in both directions parallel to the surface. The same zonation is displayed in the strobilated region and in the scolex; but in both these regions bundles of muscle fibres are well developed internally to the cortical nuclei in addition to the radial and longitudinal fibres in the cortical periphery. The cuticle has a markedly different appearance in the different regions of the larva. On the bladder the cuticle is thin (about 2 to 4 μ) and is irregularly thrown into loose folds whose contour is followed by the cortico-medullary boundary so that the cortex maintains an approximately

uniform thickness (Figs. 71 & 72). On the strobila the cuticle becomes progressively thicker anteriorly until it merges into that of the head. The body surface in the strobila region is thrown into a series of very deeply dissected ridges and grooves whose course is followed by the cuticle. The body margin thus forms a series of leaves about 60μ deep by 15μ broad (Fig. 70). The body tissue in the leaves is almost entirely cortical. The head cuticle like that of the infective stages of other species is the thickest part of the body covering and here it reaches a thickness of 10 to 15μ. Just behind the head the cuticle itself is deeply indented by a succession of grooves so that in section it has the appearance of a succession of thick blocks connected by thin basal bridges of about half the thickness of the blocks themselves. The cuticular basement boundary only slightly reflects the superficial contour (Fig. 69).

With Mallory's triple stain the triple structure of the bladder and strobila cuticle is immediately recognisable by the typical blue-red-blue succession of colours across its section. This species by this stage, therefore, recalls the cuticles of the infective stages of the other species. On the head, as in the other species, the outermost Mallory blue-staining layer is absent.

Figures 73 to 75 are electron micrographs of 60-day old bladder cuticle. The appearance in Figures 73 & 75 recalls the very similar electron micrographic

appearance of the cuticle of C. tenuicollis (Fig. 19) in that the middle layer of cuticle matrix is again composed of a granular mass of electron-dense material embedded in a less dense surrounding substance. This is a reversal from the appearance observed in the 10-day cuticle where the dispersed element was the less dense to electrons, while the more opaque substance formed the continuous element. It is possible that these differences are due to differences in fixation although the same methods were used in all cases. In the basal quarter of the cuticle there are numerous very dense bodies; these vary very much in size. The basement membrane is interrupted at intervals, and at one of these interruptions there is an undoubted tubular connection directed towards a subcuticular palisade cell. The cuticular surface is drawn out into the villous hairs or microtrichia which are typical of the other infective cysts (Figs. 73 to 75). These measure about .03μ in length and are some .05μ apart. Although Crusz (1948c) has described these microtriches in very early stages this is the earliest stage at which they have appeared in the present studies.

Histochemical tests on these cuticles produced the same results as the tests on younger cuticles with one exception, namely, with toluidine blue. There was a distinct β-metachromasia with this dye, but this was not so intense as that found in C. bovis etc.

and it was readily changed to an α-orthochromasia by 70% ethanol. This cuticle still gives a positive xanthoproteic reaction. Thus in two respects it is chemically distinct from that of C. bovis, namely, that it contains PA/S positive (Fig. 76) substances and it still contains phenolic ring substances (tyrosine, tryptophane, phenyl-alanine).

Stored foods.

At all stages of development, even as early as 10 days, glycogen (Figs. 55 & 77) and fats (Figs. 56, 57 & 79) are evident in the circumvacuolar parenchyma. The quantity of glycogen clearly increases progressively as the cyst grows and ages so that by 60 days its quantity is as great and it is as widely dispersed throughout the tissues as in the other species studied. As regards lipoids, their histochemical reactions are the same as those of the parenchymatous lipoids of the other species, but their quantity is very different. Although they are already clearly discernible at 10 days (Figs. 56 & 57) their quantity does not appreciably increase by the 60th day (Fig. 79). There would appear to be specific differences in fat metabolisms in the various species and this subject obviously merits further investigation.

DISCUSSION AND CONCLUSIONS

The arguments in favour of the particular interpretations given to the histochemical findings have been outlined already in the appropriate sections concerned with histochemical studies. The conclusions from these findings may be summarised in the statement that cysticercus cuticles in the present series show three distinct chemical types:-

1. With PA/S positive non-metachromatic polysaccharides, proteins containing arginine and amino-acids such as tryptophane etc. and phospholipids
2. With PA/S positive metachromatic polysaccharides, proteins containing arginine and amino-acids such as tryptophane etc. and phospholipids
3. With PA/S negative strongly metachromatic polysaccharides, proteins in which only arginine can be identified and phospholipids

While types 1 and 2 are found in C. fasciolaris immature and early infective stages respectively, type 3 is found in the mature infective cysts of C. bovis, C. tenuicollis and C. pisiformis. Thus while we can demonstrate a development of type 2 from type 1 in the

same species, the direct evidence is lacking which would relate type 3 to them apart from the single cyst examined of Multiceps multiceps. Unfortunately, the significance of this cyst was not appreciated until too late in the investigation, and a complete histochemical analysis was not carried out. Information is lacking concerning the nature of the proteins in this species, nevertheless in respect of its carbohydrates Multiceps multiceps provides direct information which links up types 2 and 3. In this species the cuticle of mature scolices was of definite type 3 while in the same cyst the immature scolices had a cuticle which was both PA/S positive and metachromatic. In this cyst, therefore, we have direct evidence to relate types 2 and 3, at least as regards the polysaccharide content. From the accumulated evidence, therefore, the conclusion is permissive that types 1, 2 and 3 represent successive developmental stages in the chemical composition of the cuticle. Now Hutchison (1958) has shown that 60 days is the minimum time at which C. fasciolaris can become infective to its definitive host and Rees (1951) has recorded the sizes to which the strobilocercus can grow while still in the intermediate host. The present strobilocerci were all removed at or before 60 days, and their size was considerably less than that potential for the species. It is certain, therefore, that these cysts were still capable of much greater growth and at the same time it

is quite probable that they had been arrested short of achieving infectivity. These considerations suggest the possibility that a change to type 3 was still possible in C. fasciolaris and that the failure to identify such cuticles in the present investigation was due to premature removal of the larvae. It should be noted in this connection that there is one larval tapeworm known which apparently never reaches stage 3, namely Echinococcus granulosus. The cuticle of heads and wall in this species is PA/S positive (Kilejian et al 1961). It is possible that this is related to the fact that this species is capable of an enormously extended period of growth in the intermediate host, whereas the species considered herein are not. Examination of still older C. fasciolaris cysts is obviously essential before the speculation can be extended but the possibility is suggested that a type 3 cuticle is the feature of an infective tapeworm larva, i.e. of one whose intermediate or larval growth has been terminated.

There has been, hitherto, no investigation carried out on the chemical nature of adult tapeworm cuticles and hence a critical clue to the further course of chemical development is lacking. Structurally, however, the work of Read (1955), Kent (1957), Rothman (1959, 1960) and Threadgold (1962), especially in their electron microscope studies, has provided a good basis for comparison between adult and larval

cuticles. Admittedly they worked with species (Dipylidium caninum, Raillietina cesticillus, Hymenolpeis diminuta and H. nana) which are systematically quite distinct from the present Taeniid species. Nevertheless, the striking similarity between their findings and the present leaves little room to doubt that cuticles have the same structure throughout the class of cestodes both in the adult and the infective larval stages. In all cases there is evidence for a vital continuity between the cuticular material and certain of the cortical cells; those which Threadgold has described as dark cells. The continuity cannot be denied from the present findings, and in one example at least the electron micrograph evidence (Fig. 73 and possible Fig. 10) provides positive confirmation in larval stages of Threadgolds interpretation. Here, however, we have failed to distinguish between the dark and light cortical cells which this worker describes. Threadgold and Rothman accept as fact the suggestion of Read (1955) that the discrete electron-dense bodies which are usually concentrated in the basal cuticular zone are mitochondria. The present evidence is not sufficiently good to reach so definite a conclusion. They are very inconstant structures in our present work, and although occasional examples have indications of possible cristae and double membranes their nature cannot be

definitively asserted and thus the evidence although presumptive leaves the question still undecided. On the basis of the evidence of mitochondria and of the tubular extensions from "dark cells" Threadgold considers that the cestode cuticle is vital in nature and is a modified syncytial structure and not an inanimate secreted cuticle. He refers to it as a tegument and not a cuticle. The recognition of phospholipids throughout the cuticular matrix strongly supports this view, and although the term cuticle has been employed throughout this report Threadgold's terminology is here accepted as correct.

The chemical changes of the developing cysticercus integument present an interesting analogy with those described in the insect cuticle. This when laid down consists of a neutral mucopolysaccharide, chitin with interspersed protein. At first the cuticle gives a positive PA/S reaction, but after polyphenol tanning and exposure to air, the cuticle often becomes PA/S negative although chitin is still present. Chitin is a constituent of the shell of many nematode and acanthocephalan eggs (references in von Brand 1952). Earlier workers have claimed that the tapeworm tegument contains chitin (Leuckart, 1886). Crusz (1948c) has demonstrated the presence of chitin by biochemical tests although he had denied its presence a year earlier (Crusz 1947) in the hooks of C. fasciolaris. Their

bases and the adjacent tegument, however, were alkali-soluble and hence chitin could not have been a component element there.

Holz and Pezenburg argued against the presence of neutral mucopolysaccharides on the evidence of a negative PA/S test in C. bovis cuticle, and their further test with Diaphanol/zinc chloride iodine failed to indicate chitin. No tests for chitin were performed in this study but it is assumed that chitin is not a component of cysticercus cuticles since it has not been reported by any worker concerned with the biochemical composition of tapeworms.

We have interpreted our findings on 10-day old cysts as signifying the presence of acid-mucopolysaccharides principally on the evidence on non-extinction of methylene blue staining until the pH reaches 4.1. Neutral mucopolysaccharides from pig gastric mucous for example do not stain until a pH exceeding 5 is reached (Braden, 1955). Account must be taken of the other substances present in the tegument in testing the basiphilia shown in methylene blue staining. The removal of acid-lipids does not affect the result, but there still remains in these teguments a complex of acidic and basic proteins which may contribute to the basiphilia (French & Benditt 1953) but whether their net effect is to increase or reduce it, it is not possible to decide. Nevertheless, the basiphilia of the tegument is much more pronounced than that of the parenchymatous cytoplasm in spite of

the presence in the latter of acid lipids, RNA, and acidic proteins. The conclusion that the moderate basiphilia of the tegument is due to acid mucopolysaccharides, therefore, appears justified. This argument might be carried further to suggest that the earliest development of the peripheral cytoplasm to form a cystic tegument is represented by a physical concentration, and hence the increase in opacity to electrons, together with an accumulation of acid mucopolysaccharides (and acid lipids). At this stage the acidity is due to carboxylic groups, and the substance remains PA/S positive and non-metachromatic. As stage 2 approaches, such as we see in 60-day old C. fasciolaris and immature scolices of Multiceps multiceps, the acidity increases to produce a metachromasia and this may be due to the incorporation of phosphoryl and/or ester sulphate groups (10-40 day old cysts become metachromatic following experimental sulphation).

It is assumed that the appearance of metachromasia with toluidine blue is due to an increase in acidity of the mucopolysaccharides. Nevertheless, it must be admitted that this is not accompanied by a significantly increased basiphilia as indicated by methylene blue extinction. The end point for methylene blue staining was the same for all ages of C. fasciolaris up to 60 days.

The change from stage 2 to 3 appears quite definitely to be associated with increased sulphation of the mucopolysaccharides since there is a loss of metachromasia following methylation and subsequent demethylation in stage 3 teguments. This change, however, is associated with two others, namely loss of PA/S reactivity and a change in the amino-acid composition. Negative Millon and Xanthoproteic tests, although these are not entirely satisfactory tests, imply a change in or loss of the Tyrosine, Tryptophane types of amino acid, while the continued presence of arginine argues in favour of basic protein (hence the strong acidophilia with eosin). It is conceivable that condensation of these with the basiphilic acid mucopolysaccharides is the factor which eliminates the PA/S reactivity, and this condensation is strong enough to resist separation by sodium hydroxide. Of all the changes occurring between stages 2 and 3 it would appear that the change in the protein components is the most fundamental. Such a change could be the result of a tanning process but present evidence permits no more than to raise the suggestion. It is interesting that the most radical changes in composition of the tegument occur between stages 2 and 3 when the cyst is changing from its active larval growing stage to enter the infective stage where the physiological problems to be solved are of quite a different kind.

The nature of the cestode integument, its origin and relations to other tissues has been a continuous subject for debate and speculation since the earliest days of microscopy. Young (1935) in reviewing earlier opinions points out that the continuing uncertainty has been due partly to the fact that much information has accumulated incidentally to other studies, and to the very real difficulties that these organisms present as histological subjects. A continuous awareness of the peculiar role of the cestode cuticle has encouraged far-reaching interpretations of often insufficient findings. In addition to the usual mechanical and protective role the cuticle of a gut-inhabiting cestode has to fulfil an absorptive function and an enzyme-resisting function. These two latter appear as almost antithetic roles. To explain the last, opinions have either tended to regard the cuticle as 'horny' and indigestible, or to rely on a hypothetical antienzyme system. Threadgold (1962) has pointed out that neither of these views is necessary if it be accepted that the cuticle consists of living material. Digestion by host enzymes of the superficial layers could continue, and the parasite could survive by a continuous process of replacement. Turning to the cysticercus, it is obvious that they are not exposed to the enzyme rich medium such as bathes an adult tapeworm. They do, however, have to overcome the hazard of close investment by macrophages and eosinophils in their early

developmental period. The sections studied of 10-day old C. fasciolaris show a cuticular surface which is closely attacked by host cells, and which in places is distinctly ragged and broken up. There is even a tendency for cuticles at this stage to desquamate or even slough completely. Assuming such examples to be normal, and not cases of early degeneration, the larval cuticle would appear to provide support for Threadgold's view of continuing erosion and replacement. As cysts become older the macrophage attack is overcome and the cyst enjoys insulation through the fibrous capsule which develops. While this closely invests the parasite at first, the parasite later withdraws (e.g. C. fasciolaris at 60 days) and now the surface characters become quite distinct.

The infective larva of a cysticercus after ingestion by the definitive host has to overcome two hazards, first the acid peptic fermentation of the stomach, and later the alkaline trypsins in the intestine. During this passage, the bladder is digested and in the case of C. fasciolaris much of the strobilated region is digested while the scolex resists digestion to become established and give rise to the adult tapeworm. Chemically, there has been no difference identified between bladder and scolex cuticles which could account for their differential behaviour in relation to digestive enzymes. Crusz (1948c) has shown that the cuticle of C. fasciolaris head is digestible in vitro by both pepsin and trypsin - incidentally indicating the absence of both chitin and

keratin. Hence it is improbable that resistance to proteolytic enzymes is a function of the chemical property of the cuticle, but must be a manifestation of some vital function. The only significant difference between bladder and head cuticles lies in the very marked difference of thickness between them. It is noteworthy that calcium salts are deposited abundantly in the head but not significantly in the bladder tissues, and these may assist the head to complete its passage through the hostile stomach medium whereas the bladder lacks this assistance. (von Brand (1952) suggests simply that the calcareous corpuscles may serve as an alkali reserve). On balance these considerations conform well with the view of Threadgold (1962) and Watson (1960) that the adult tapeworm tolerates the digestive activity by a process of erosion and replacement. A thick cuticle would be better adapted for this than a thin one such as the bladder possesses.

In connection with the question of the loss of the bladder in the adult host it is worth mention that the bulk of the stored food material of the cysticercus occurs in the bladder medulla. The function of the fat and glycogen must, therefore, be to maintain the larva in its later stages in the intermediate host when either its own cuticle has a diminished absorptive capacity or its access to food materials has been limited by the host capsule. In any case it is well-known that the

cysticercus has only a limited life span in the intermediate host, and will die if it does not gain access to the definitive one. Evidently the scolex has no need for great food reserves after it has been separated from the bladder in the gut of the definitive host.

With regard to the absorptive function most workers have been impressed by the superficial resemblance between the cestode microtriches and the villi of intestinal cells. Such processes obviously greatly increase the surface area and are considered by most authors to serve in such a manner to assist absorption. Two views have been held; the first that they are protoplasmic processes passing through the cuticle and arising in the subcuticular cells (Scheifferdecker, 1874: Young, 1908: Pratt, 1909: Crusz, 1948, and Holz and Pezenburg, 1957 inter alia). The alternative is that they are true cuticular processes (Leuckart, 1886: Voge, 1962). Leuckart attached no importance to the microtriches and considered them as "the remains of an older exfoliated and altered cuticle". He supported his argument for their non-absorptive role by their absence on bladder cuticle and abundant presence within the invaginated head canal. In these observations he was clearly erroneous. Recently electron microscope studies have fully confirmed the view that the microtriches are cuticular processes, the

published figures leaving no room for doubt (Read, 1955: Kent, 1957: Threadgold, 1962 and Siddiqui, 1962). Rothman, 1959 and 1960, has described the same findings although he has not published his electro micrographs.

The microtriches as found by Kent, Rothman and Read in Hymenolepis and Raillietine species contain a central vacuole. Those found in Dipylidium caninum by Threadgold and in all 4 species of cysticercus studied by electron micrography in the present investigation contained a central core of equivalent electron density to the cuticular matrix. It is impossible to decide whether these differences are real or artificial, the question, like the comparable one of reversed electron densities of continuous and dispersed elements in different cysticercus species, remains an open one. Threadgold has drawn attention to the dense distal terminations of the microtriches and suggests for them two other possible functions

1. to retain position by interdigitation with the intestinal villi of the host, and
2. as abrasives to destroy host cells and thereby increase the available nutrients.

It is true that both Threadgold's and Kent's micrographs show villi of varying electron density, but it is not convincingly demonstrated that the density at the apices is always greatest. Varying density is shown in the present work and Figure 19 of C. tenuicollis illustrates this clearly. Here, however, the microtriches appear

to be densest about their middle. The pointed extremities which appear in Kent's and Threadgold's figures appear more likely to be the result of oblique sectioning than that this is their actual shape. It should be recalled that Goodchild and Wells, 1957, found a very close parallel between the amino-acid content and ratios of tissues of Hymenolepis diminuta and the mucosal cells of rat intestine indicating a direct source of protein precursors from the host cells possibly as Threadgold suggests through microtrichial damage.

Another feature which has been claimed by some authors to increase the absorptive capacity of the cuticle is the presence of fine canals penetrating the cuticular substance from the exterior to the basement. These pore-canals were first described by Sommer & Landois (1872) and have been observed by many workers although Young (1908) has denied their presence. Among recent workers Rothman (1959) describes them in Hymenolepis diminuta adults and Threadgold (1962) describes and figures them in Dipylidium caninum. Among the micrographs in the present work a canal opening on the surface was only observed once in C. fasciolaris (Fig. 53) and it appears that they are rare in the cuticles of cysticercus. It is interesting to note that Young who was working with C. pisiformis never observed pore canals, and that Holz & Pezenburg (1957) who worked with C. bovis also failed to refer to them.

The abundant occurrence of phospholipids at all stages of cuticle development is a notable feature. The structure of this group of lipoids with their acidic and basic groups gives them peculiar properties among the lipoids. They behave as zwitterions and moreover have both hydrophilic and hydrophobic properties and probably have a functional role in the absorption mechanism of the cuticle especially in fat translocation (Hedrick, 1958). Von Brand (1952) and Smyth (1961) have reviewed the various chemical analyses carried out on whole tapeworms and the evidence is that phospholipids form a very high proportion of the lipoid material - up to 30% in Diphyllobothrium latum (Totterman & Kirk, 1939). The same is true of larvae, for example in C. fasciolaris phospholipids represent 30 per cent of the total lipoid material according to Salisbury & Anderson (1939).

Among earlier workers Minckert (1905) and Young (1908) have described cysticercus cuticles as composed of multiple layers. Minckert recognised three, and Young considered that the basement membrane was not a cuticular element and the cuticle, therefore, contained only 2 layers. More recently Crusz (1948) and Voge (1962) have asserted that the cuticle is a monolayer structure. In both cases they found the cuticle unicolorous blue-stained with Mallory's triple stain. This result is quite different from the present findings with Mallory's stain in which the bulk of the cuticle stained red, and

this was bounded internally and externally by thin blue layers. Presumably this difference is due to technique: according to Pantin (1948), differences in timing and procedure with Mallory's stain produce great variations in result with different tissues and different brands of stain. Voge (1962) identified a thin blue staining layer below the cuticle and this probably corresponds to the basal blue layer of the present work. Holz and Pezenburg (1957) positively deny the existence of a subcuticular basement membrane, and describe a continuity between cuticule and underlying cells. Electron microscopy confirms them in this last particular, but it also reveals a basement and a superficial thin layer.

We have referred loosely to the lipoid droplets in the parenchyma as food reserves. Reid (1942) has shown, however, that Raillietina cesticillus contains equal amounts of fat whether taken from nourished or starving chicken hosts, although in the second the parasite had depleted its glycogen reserves. Smyth (1949) has found that the fat increases in plerocercoid larvae of Ligula intestinalis which are starved. Stephenson (1947) showed that the fluke Fasciola hepatica actually excretes fat as a waste product although in the same site the fluke Dicrocoelium dendriticum according to von Brand (1934a) does not. Von Brand (1952) points out that lipoids are unsuitable substrates for anaerobic metabolism and considers it probable that accumulation

of fat represents an end point in the degradation of carbohydrates. It is significant that we have seen phospholipids in the excretory channels of cysticerci. These, which are readily translocated, may represent excretory products and it is even possible that the phospholipids concentrated in the tegument may also be waste products which are lost in the erosion - regeneration process postulated by Threadgold (1962).

SUMMARY

1. The cuticles of the mature cysticerci of the Taeniid tapeworms Taeniorhynchus saginatus, Taenia pisiformis and Taenia hydatigena, and of 10-day, 20-day, 40-day and 60-day old cysticerci and strobilocerci of Hydatigera taeniaeformis were studied by histological, histochemical and electron-microscopic techniques. Supplementary histological and histochemical observations were made on the cysticercus of Taenia solium and the coenurus of Multiceps multiceps.

2. Electron and photomicroscopic methods defined the cuticle as a three layered structure on the bladder of mature specimens of all species studied. The cuticle is covered with fine villi of microtriches composed of the middle and superficial component layers. The cuticle on the head was from 10 to 20 times as thick as bladder cuticle but it lacked both the external layer and the microtriches. The cuticle was found to be in continuity with the underlying cortical cells, which in the youngest forms are completely inseparable from the cuticle. Connection in older individuals is mediated by pseudopodial connection tubes.

3. The chemical composition of the cuticle changes with age and three distinct types are recognised which are considered to represent age changes. In the earliest stage acid mucopolysaccharides, proteins and phospholipids are present and the cuticle is PA/S positive and non-metachromatic. In the second phase the cuticle is still PA/S positive but is now metachromatic and this is taken to indicate the occurrence of more strongly acid groups in the mucopolysaccharides. In the third step which is taken to represent the infective non-growing final larval stage, sulphate ester groups occur in the mucopolysaccharides and the protein element has undergone a change suggestive of tanning. The cuticle is now PA/S negative, strongly metachromatic, and strongly basiphilic.

4. It is argued that the cuticle is a vital tissue and not a cellular secretion. The bodies described by other authors as mitochondria were recognised but their nature was not positively identified.

5. The implications of these findings for the physiology of the tapeworm are discussed.

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TABLE I

HISTOCHEMICAL STUDY OF THE CYSTICERCUS

Test	Results			
	Cuticle			Parenchyma
	C.b.	C.t.	C.p.	C.b..C.t..C.p.
Safranine (0.5% aqueous) for basiphilia	++	++	++	++
Eosine (0.5% aqueous) for acidophilia	++	++	++	++
Himes & Moriber triple stain	yellow	yellow	yellow	red
PA/S (Hotchkiss)	-	-	-	++
PA/S (after Hotchkiss)	-	-	-	++
PA/S (McManus)	-	-	-	++
PA/S after NaOH (Hale)	-	-	-	++
Alcian blue neutral medium	++	++	++	++
Alcian blue acid medium pH 2.6	++	++	++	++
Methylene blue extinction				
pH 8.1	+++	+++	+++	+++
pH 7.2	+++	+++	+++	+++
pH 6.1	+++	+++	+++	+++
pH 5.3	++	++	++	++
pH 4.1	++	++	++	++
pH 3.8	++	++	++	+
pH 2.2	-	--	-	+
Metachromasia (Toludine blue)	+++	+++	+++	+++
Metachromasia after methylation	-	-	-	-
Metachromasia reversal NaOH	+	+	+	+
Alcian blue after 48 hrs. and- 6 hrs. methylation at room and 60°C. respt.		-	-	-
Methylene blue after methylation				
pH 7.4	++	++	++	++
pH 4.1	+	+		
pH 2.1				

Test	Results			
	Cuticle			Parenchyma
	C.b.	C.t.	C.p.	C.b..C.t..C.p.
Muci haematein	++	++	++	++
Colloidal Iron (Hale)	++	++	++	++
Best's Carmine	++	++	++	++
Best's Carmine after malt diastase	++	++	++	++
PA/S after malt diastase	-	-	-	-
Millons (Baker) Hg/nitrite	-	-	-	+
Xanthoproteic	-	-	-	-
Sakaguchi	+++	+++	+++	++
Feulgen	-	-	-	++
Pyronine/methyl green	++	++	++	++
Pyronine/methyl green after ribonuclease	++	++	++	+
Sudanophilia				
Sudan Black B	++	++	++	+++
Sudan IV	++	++	++	+++
Sudan acetylated	++	++	++	+++
Sudan Black B after cold acetone	++	++	++	-
Sudan Black B after hot pyridine	-	-	-	-
Nile blue sulphate	blue	blue	blue	blue
Acid haematein for phospholipids	+++	+++	+++	++
Acid haematein for phospholipids after hot pyridine	-	-	-	-
Performic acid/Schiff	+	+	+	+
Performic acid/Schiff after bromination	-	-	-	-
Leiberman	-	-	-	-
Cobalt method for calcium	-	-	-	+ (Calcarious corpuscles in head region)

Test	Results			
	Cuticle			Parenchyma
	C.b.	C.t.	C.p.	C.b.,C.t.,C.p.
Cobalt method after treatment 2% Nitric acid	-	-	-	-

+++ = strong reaction

++ = moderate reaction

+ = weak reaction

- = negative reaction

C.b.= Cysticercus bovis

C.t.= Cysticercus tenuicollis

C.p.= Cysticercus pisiformis

TABLE II

HISTOCHEMICAL STUDY OF THE DEVELOPING CYSTICERCUS FASCIOLARIS

Test	Results				
	Development of Cuticle in days				Parenchyma
	10	20	40	60	
Safranine (0.5% aqueous) for basiphilia	++	++	++	++	++
Eosin (0.5% aqueous) for acidophilia	++	++	++	++	++
Himes & Moriber Triple stain	orange	orange	orange	orange	red
PA/S (McManus)	++	++	++	++	++
PA/S control with acetic anhydride	-	-	-	-	-
PA/S control reversal NaOH	++	++	++	++	++
PA/S after malt diastase	++	++	++	++	-
PA/S after deamination	++	o	o	++	++
PA/S after hot pyridine extraction	++	o	o	++	++
Alcian blue neutral medium	+	+	+	+	+
Alcian blue acid medium pH 2.6	+	+	+	+	+
Methylene blue extinction					
pH 8.1	++	++	++	++	++
pH 4.1	+	+	+	+	+
pH 2.1	-	-	-	-	-
Metachromasia (Toluidine blue)	-	-	-	+	+
Metachromasia (after sulphation)	++	++	++	++	+
Best's Carmine	++	++	++	++	+++
Best's Carmine after malt diastase	++	++	++	++	-

Test	Results				
	Development of Cuticle in days				Parenchyma
	10	20	40	60	
Xanthoproteic	+	+	+	+	+
Sakaguchi	++	++	++	++	++
Feulgen	-	-	-	-	++
Pyronine/methyl green	++	++	++	++	++
Pyronine/methyl green after ribonuclease	++	++	++	++	++
Sudanophilia					
Sudan Black B	++	++	++	++	++
Sudan IV	++	++	++	++	++
Sudan acetylated	++	++	++	++	++
Nile blue sulphate	blue	blue	blue	blue	blue
Sudanophilia after acetone	++	++	++	++	-
Sudanophilia after pyridine extraction	-	-	-	-	-
Acid haematein for phospholipids	++	++	++	++	++
Acid haematein for phospholipids after hot pyridine extraction	-	-	-	-	-
Performic acid/Schiff	+	o	o	+	+
Performic acid/Schiff after bromination	-	o	o	-	-
Lieberman	-	-	-	-	-
Cobalt method for calcium	-	-	-	-	+ (Calcarious corpuscles over head in 60 dys. only)
Cobalt method after treatment with Nitric acid 2%	-	-	-	-	-

+++ = strong reaction; ++ = moderate reaction

\+ = weak reaction; - = no reaction

o = not done

APPENDIX I

Summary of Histochemical Tests

1. Basiphilia

   Stained in 9.5% Aqueous safranin for 20 mins., rinsed Aq.dist. dehydrated in graded Iso-butyl alcohol cleared in Xylene and mounted in D.P.X.

2. Acidophilia

   As in 1, but stained 0.5% Eosin yellow for 20 mins.

3. Himes triple stain

   Hydrolyzed 12 mins. in 1N HCl at 60°C; stained 5 mins. in azure A-Schiff solution and rinsed; bleached in 2 washes for 2 mins. each in acid metabisulphite solution; immersed in periodic acid solution for 7 mins. and rinsed; stained 20 mins. in basic fuchsin Schiff and bleached 2 mins. each in 2 acid metabisulphite washes; stained 2 mins. in 0.02% Naphthol yellow S and rinsed. Dehydrated in tertiary butanol 2 changes of 2 mins., cleared in Xylene and mounted in D.P.X.

4. Periodic acid Schiff tests

   (a) McManus. Immersed for 5 mins. in 0.5% aqueous periodic acid and washed for 5 mins. in running water. Stained Schiff reagent (Appendix II B 2 a Sol. II) for 15 mins. then rinsed in three changes freshly prepared sulphite rinse 2 mins. each, washed water, dehydrated in graded ethanols, cleared in Xylene and mounted

(b) Intensified Hale P.A.S. (1953)

(c) Hotchkiss. Immersed for 5 mins. in alcoholic periodic acid and washed in 70% ethanol. Immersed in reducing agent (Appendix II 2 b Sol. III) for 5 mins. and washed in 70% ethanol. Stained Schiff reagent (as in McManus), then rinsed in 3 changes of sulphite rinse dehydrated etc.

(d) After Hotchkiss

From absolute ethanol sections immersed in 1% Celloidin in equal parts ethanol and ethyl ether. After air drying washed in 70% ethanol and passed to alcoholic periodic acid and remaining procedure as for Hotchkiss except that Schiff reagent is prepared differently (Appendix II B 2 c)

Note. The P.A.S. Tests involved the same main procedures but differ in so far as the McManus test does not employ a reducing agent between the periodic and Schiff steps the periodic acid is aqueous. The Hale intensification procedure 4(b) involves treatment of the section with 0.2N Sodium hydroxide for 15 mins. before periodic acid treatment which is now extended to 15 mins. and exposure to Schiff reagent is doubled to 30 mins. The "After Hotchkiss" method differs from the Hotchkiss in that it is carried out on celloidin impregnated

sections, and the Schiff reagent has the dye much less concentrated and contains Thionyl chloride in place of Hydrochloric acid.

5. P.A.S. after blocking and extraction

(a) Acetylation on materials already shown P.A.S. positive. After passing to water section placed in Acetic anhydride/pyridine for 45 mins. at R. temp. Then washed in water before carrying out P.A.S. test.

(b) Deacetylation of acetylated sections.

After acetylation material saponified by 1N Potassium hydroxide for 45 mins. then P.A.S. test carried out after washing.

(c) Deamination.

From water section treated van Slyke's reagent overnight (12 hrs.) and washed before P.A.S. testing (Appendix II b 11).

(d) Pyridine extraction

Material transferred to Pyridine at 60°C for 24 hrs. after 20 hrs. fixation in weak Bouin and passed through graded Ethanols (70% and 50%) to water. After 2 hrs. washing in running water sections cut from gelatine embedded blocks on freezing microtome. P.A.S. technique carried out on sections.

6. Chromic acid/Schiff test.

Sections from water oxidized in 5% aqueous chromic acid for ½ hr. washed in running water for 5 mins. and Schiff staining carried out as for McManus test.

7. Best's Carmine

(Appendix II b 13, 14) Sections celloidin impregnated as in "After Hotchkiss" (4c above) and passed to 70% ethanol, and stained Best's carmine (Appendix II 13) for 15 mins. After passing to 96% ethanol sections differentiated in a mixture of ethanol, methanol and distilled water 8: 4: 10 by volume, celloidin removed by ethanol-ether mixture (equal parts by vol.) then via ethanol absolute to D.P.X. mountant. Some sections counterstained with Ehrlich's haematoxylin after differentiation.

8. Malt-diastase digestion

Sections passed from water to Malt-diastase solution 0.1% in Phosphate buffer (Appendix II c 2) at 37°C for 1 hour. Control sections in buffer alone. Best's carmine and P.A.S. tests carried out on treated and control sections.

9. Metachromasia

Sections from water stained 0.5% aqueous toluidine blue for 2 mins. and examined in water immediately after washing. Section then mounted by usual procedure in D.P.X.

10. Metachromasia after blocking

(a) Methylation.

After 2 changes absolute ethanol sections washed twice in absolute methanol then immersed in methylation reagent (Appendix II b 4) for 4 hours. Control sections treated with methanol alone. After washing in 70%

ethanol sections taken to water then Metachromasia test carried out.

(b) Demethylation of methylated sections

As for deacetylation then Metachromasia test performed.

11. Alcian blue test

Method I Neutral (Steedman) sections from water stained for 10 seconds in 1% aqueous Alcian blue then washed and mounted in D.P.X. after appropriate procedure.

Method II acidic (McManus) sections transferred from water to 3% acetic acid for 2 mins. then stained 1% alcian blue in 3% acetic acid for 30 mins. mounted in D.P.X. after washing for a few minutes in running water.

12. Methylene blue extinction

From water sections are stained in 0.25% Methylene blue in various veronal buffers (Appendix II c 1) for 24 hours at room temperature. After washing rapidly in distilled water sections examined in distilled water to determine pH. end point.

13. Sulphation

Sections air dried from Benzene then placed for 10 mins. in sulphation medium (either Cone sulphuric and Glacial acetic acid equal parts or Sulphuryl chloride). From acid mixture sections passed to water through graded solutions, and from Sulphuryl chloride sections passed to benzene direct and impregnated with celloidin before passing to water via graded ethanols, stained in buffered Methylene blue (Appendix II B 5) for 30 mins. then rinsed in distilled water. Appropriate procedure to D.P.X.

mounting following the celloidin being removed where present.

14. Feulgen test for D.N.A.

From water, sections hydrolysed in preheated 60°C 1N HCl for 10 mins. After rinsing in distilled water stained Schiff reagent (Appendix II B 2a) for 1½ hrs. Washed in sulphite rinse then water. Sections mounted in D.P.X. with or without counterstaining in 0.25% light green in 70% ethanol for 15 seconds sections compared with controls in which HCl replaced by water.

15. Pyronin/Methyl green test for R.N.A.

Sections removed from water and surplus water removed by blotting with filter paper. Stained ½ hour in reagent (Appendix II B 6) then rinsed distilled water and blotted nearly dry. Dehydrated in acetone 1 min. and then mounted via acetone/xylene and xylene in D.P.X.

16. Pyronin/Methyl green after ribonuclease

Sections from water incubated for 3 hrs. at 60°C in preheated freshly prepared enzyme solution (Appendix II D). After washing in running water for 1 min. sections are blotted dry before staining with Pyronin/Methyl green.

17. Sakaguchi test (Fixation in Zenker) for Guanidine derivatives.

Sections celloidin coated then transferred through 90% to 70% ethanol, washed in Iodine 0.5% in 70% ethanol then 5% aqueous sodium thiosulphate to remove mercury precipitate (from Zenker fixative). After washing slides flooded with 2 ml Sodium hydroxide then 2 drops

L-naphthol 1% in 70% ethanol and 4 drops milton (Sodium hypochlorite 1%). After 15 mins. slides blotted and transferred to pyridine chloroform mixture 75:25 by vol. Examination done in pyridine - chloroform temporary mountant.

18. Millon test (Fixation Formol saline and embedded in celloidin)

Sections from water transferred to Millons reagent 5 ml plus 0.5 ml Sodium nitrite (solution II Appendix II B 7) and boiled. After cooling washed in 3 changes distilled water - 2 mins. each, and mounted in glycerine jelly or Farrant's medium.

19. Xanthoproteic test (Fixation in Zenker)

Section blotted to remove surplus Xylene concentrated Nitric acid added and section examined, yellow coloration indicates tyrosine.

Second section after Nitric acid washed with distilled water and section exposed to Ammonia vapour. Orange colour when examined indicates Tryptophane.

20. Sudanophilia

Sections brought from water through 50% to 70% Ethanol, stained Sudan black or Sudan acetylated for 1 min; or Sudan IV for 2½ mins. then washed 5 secs. in 70% Ethanol and returned through 50% Ethanol (1 minute) to water. Counterstained Mayer's Carmalum for 2½ mins. and mounted Farrant's medium after washing in distilled water.

21. Sudanophilia after extraction

(a) Pyridine. Sudanophilic test carried out on pyridine extracted section prepared as in 5 d above.
(b) Acetone. Fresh material fixed and extracted in cold acetone for 24 hrs., then embedded gelatine and cut frozen section. Sudanophilic tests then applied.

22. Nile blue sulphate

Sections from water stained 1% aqueous Nile blue sulphate at 60°C for 5 mins. then rapidly washed water at 60°C. Differentiated in 1% Acetic acid at 60°C for 30 sec. and after washing mounted in Farrant's medium. (Section A).
A second section (Section B) restained for 5 mins. in Nile blue sulphate 0.02% at 60°C after first differentiation and washing then again differentiated and washed as above before mounting.

23. Acid haematein test

After fixation for 6 hours on Formaldehyde calcium (Appendix II A 9) the material post-chromed without washing (Potassium dichromate 5% and Calcium chloride 1%) for 18 hours at room temperature. Transferred to second Dichromate-calcium bath at 60°C for 24 hrs. After washing for 6 hrs. material embedded in 25% gelatine and sections cut by freezing microtome. Sections again chromed at 60°C for 1 hour and washed in several changes of water finishing in distilled water. Stained freshly prepared Acid haematein (Appendix II B 9)

for 5 hrs. at 37°C, washed and transferred for 18 hrs. in Borax-ferricyanide (Appendix II B 9) at 37°C, mounted Farrant's medium after washing in several changes in water.

24. Acid haematein after Pyridine extraction

Control sections fixed weak Bouin and extracted in hot pyridine (as in 5 d above) before applying Acid haematein process.

25. Liebermann's Test

Gelatine sections mordanted for 1 day in 2.5% Iron alum at 37°C then rinsed and blotted dry on slide. A few drops of reagent 2 (Appendix II B 10) added and examined directly.

26. Cobalt chloride test for Calcium

Sections from water stained in 3% aqueous Cobalt chloride for 15 mins. and rinsed distilled water. Ammonium sulphide solution (10 drops strong sol. in 100 mls water) added for 1 min. after washing counterstained where required 0.1% aqueous Eosin, and rinsed distilled water. Mounted D.P.X. after appropriate procedure.

27. Cobalt chloride after Calcium extraction

Control sections transferred from water to 2% Nitric acid for 12 hrs. then after washing procedure followed as in 26 above.

APPENDIX II

Formulation of reagents used

A. Fixative Solutions

1. Alcoholic Formalin

Formalin (40% Formaldehyde)	100 ml
Ethanol 96%	900 ml
Calcium acetate	0.5 gm (to ensure neutrality)

2. Carnoy (6:3:1)

Ethanol absolute	60 ml
Chloroform	30 ml
Acetic acid (glacial)	10 ml

3. Picro-alcoholic formalin (Gendre's fluid)

Saturated Picric acid in 96% Ethanol	85 ml
Formalin	10 ml
Acetic acid (glacial)	5 ml

4. Bouin

Saturated Picric acid in water	75 ml
Formalin	25 ml
Acetic acid (glacial)	5 ml

5. Weak Bouin

Saturated aqueous Picric acid	50 ml
Formalin	10 ml
Acetic acid (glacial)	5 ml
Distilled water	35 ml

6. Zenker

Mercuric chloride ..... 5 gm

Potassium dichromate ..... 2.5 gm

Sodium sulphate ..... 1 gm

Distilled water ..... 100 ml

1 ml glacial Acetic acid added to 20 ml solution immediately before use (Ph = 2.3)

7. Helly

Solution as for Zenker

1 ml neutralized Formal added to 20 ml solution immediately before use (Ph = 4.7)

8. Flemming

Chromic acid 1% in water ..... 15 ml

Osmium tetroxide 2% in water ..... 4 ml

Acetic acid (glacial) ..... 1 ml

9. Formaldehyde Calcium

Formalin ..... 10 ml

Calcium chloride (anhydrous) 10% in water ... 10 ml

Distilled water ..... 80 ml

10. Formal-saline

Formalin ..... 10 ml

Sodium chloride 10% in water ..... 7 ml

Distilled water ..... 83 ml

11. Formaldehyde methanol pyridine

Formalin ..... 2.5 ml

Methanol ..... 20 ml

Pyridine ..... 0.5 ml

Water added to make 25 ml

12. Ethanol-acetone

Equal parts v/v Ethanol absolute and acetone

B. Histochemical reagents

1. Himes method (Test 3) all solutions freshly prepared.

Solution I. Azure A (Gurr) ..... 0.5 gm
Bleach (Sol. II) ..... 100 ml

Solution II. Bleach
1N Hydrochloric acid .... 5 ml
10% Potassium metabisulphite aqueous ... 5 ml
Distilled water ..... 90 ml

Solution III. Periodic acid ..... 0.8 gm
Water ..... 90 ml
0.2M Sodium acetate aqueous ... 10 ml

Solution IV. Schiff reagent McManus Vide infra

Solution V. Naphthol yellow S ..... 1 gm
1% Acetic acid ..... 100 ml

Used 2 ml in 100 m 1% Acetic acid.

2. Periodic Schiff tests

(a) McManus (Test 4a)

Solution I. Periodic acid ..... 0.5 gm
Water ..... 100 ml

Solution II. Schiff reagent (also used in Hotchkiss & Feulgen Test)

Basic Fuchsin ..... 1 gm
Boiling distilled water ..... 200 ml

1N Hydrochloric acid 20 ml added after cooling to 50°C and filtration

Sodium metabisulphite 1 gm. added after cooling to 25°C and filtration

Activated charcoal 1 gm added after 48 hrs. at room temperature in dark.

Solution stored at 4°C after filtration

(b) Intensified P.A.S. Hale 1953, (Test 4b)

McManus P.A.S. used

(c) Hotchkiss (Test 4c)

Solution I. Periodic acid ..... 0.4 gm
Ethanol absolute ..... 35 ml
0.2M Sodium acetate aqueous ... 5 ml
Distilled water ..... 10 ml

Solution II. Schiff as for McManus (Test 4a)

Solution III. Reducing solution
Potassium iodide ..... 1 gm
Sodium thiosulphate .... 1 gm
Ethanol absolute ..... 30 ml
2N Hydrochloric acid ... 0.5 ml
Distilled water ..... 20 ml

(The sulphur deposit which forms is ignored)

Solution IV. Sulphite rinse
As for Himes Solution II (Bleach)

(d) After Hotchkiss (Test 4d)

Solution I. As for Hotchkiss Solution I.

Solution II. Schiff reagent (Barger and Delamster, 1948)

Basic Fuchsin ..... 1 gm

Boiling Distilled water .. 400 ml

Thionyl chloride ... 1 ml added after cooling to 50°C and filtration

Activated charcoal ... 2 gm added after 12 hrs. in dark.

Solution stored at 4°C after filtration

Solution III and IV as for Hotchkiss III and IV above.

3. Acetylation reagent (Tests 5a and b)

Anhydrous pyridine ..... 24 ml

Acetic anhydride ..... 16 ml

4. Methylation reagent (Tests 10a and b)

Hydrochloric acid cone ..... 0.3 ml

Methanol absolute anhydrous ..... 40 ml

5. Methylene blue reagent following sulphation (Test 13)

Dipotassium hydrogen phosphate 0.1M Sol .. 8 ml

0.1N Hydrochloric acid ..... 4 ml

Methylene blue (Gurr) 1% ..... 2 ml

Distilled water ..... 26 ml

6. Pyronin/methyl green reagent (Test 15)

Acetate buffer (Appendix II c 3) ..... 50 ml

Pyronin 0.5% aqueous ..... 37 ml

Methyl green 0.5% aqueous (chloroform extracted) 13 ml

7. Millon reagent (Test 18)

Solution I. Sulphuric acid (conc.) ... 10 ml added to

Distilled water ..... 90 ml

Mercuric sulphate ..... 10 gm

dissolved by heating

Distilled water add 200 ml after cooling

Solution II. Sodium nitrite 0.25% aqueous

8. Sudan IV (Test 20a)

Ethanol 70% ..... 50 ml

Acetone ..... 50 ml

Sudan IV ..... to saturation

9. Acid haematein (Test 23)

Solution I. Haematoxylin (BDH Ph reagent) 0.05 gm

Distilled water .... 48 ml

Sodium iodate 1% aqueous 1 ml exactly heated to boiling point

Acetic acid (glacial) 1 ml added after cooling freshly prepared

Solution II. Potassium ferricyanide 0.25 gm

Borax (powdered Na-tetraborate 10 H_2O) 0.25 gm

Distilled water ..... 100 ml

kept in dark

10. Leibermann reagent (Test 25)

Solution I. Iron alum 2.5% aqueous

Solution II. Conc. Sulphuric and glacial acetic acid equal volumes

11. Deamination (Von Slyke's nitrous acid reagent) (Test 5c)

Sodium nitrite 6 gm. in water 10 ml

Acetic acid (glacial) ..... 5 ml

Distilled water ..... 25 ml

12. Cobalt chloride reagent (Test 26 and 27)

Solution I. Cobalt chloride 3% aqueous

Solution II. Ammonium sulphide 10 drops (strong sol.)

Distilled water ..... 100 ml

13. Best's Carmine (Test 7)

Carmine ..... 2 gm

Potassium carbonate ..... 1 gm

Potassium chloride ..... 5 gm

Distilled water ..... 60 ml

Used two parts stock solution to 3 parts

Ammonia 880 and 3 parts Methanol

14. Best's differentator (Test 7)

Ethanol absolute ..... 80 ml

Methanol absolute ..... 40 ml

Distilled water ..... 100 ml

C. Buffer Solutions

1. Veronal buffers for Methylene blue extinction test (Test 12)

Stock solution

Sodium diethylbarbiturate (Veronal) ... 14.714 gm

Sodium acetate ..... 9.14 gm

Distilled water ..... add 500 ml

Buffer mixture

V.A. Stock Solution	0.1N Hydrochloric acid	Distilled Water Add	Ph
	16.0		2.6
	13.0		3.9
	12.0		4.1
5 ml	8.0	23 ml	5.3
	7.0		6.1
	5.5		7.3
	2.0		8.2

2. Phosphate buffer for Malt diastase digestion (Test 8)

Disodium hydrogen phosphate (anhydrous) ... 0.28 gm

Monosodium dihydrogen phosphate ..... 1.97 gm

Sodium chloride ..... 8.00 gm

Thymol (trace) as preservative

Distilled water (freshly boiled) 1000 ml

3. Acetate buffer for Pyronin/methyl green test (Test 15)

0.2N Acetic acid ..... 81 ml

0.2M Sodium acetate ..... 119 ml

Ph = 4.8

D. Enzyme (Ribonuclease Test 16)

Preparation of Ribonuclease (Bradbury 1956) (16)

About 40 ml Human saliva gradually heated to 80°C in a water bath and maintained at this temperature for 10 mins. This destroys all enzymes except ribonuclease which is relatively heat stable (Pearse 1953). The saliva is

then cooled and centrifuged for 5 mins. to remove the bulk of the mucus. The opalescent; viscous supernatant is used as the enzyme reagent.

STUDIES ON CYSTICERCI OF THE GENUS TAENIA.

I. The cuticle in the cysticerci of *T. saginata*. *T. hydatigena*, and *T. pisiformis*.

by E.H. Siddiqui,

(Sub-Department of Parasitology, Department of Zoology, University of Edinburgh.)

With three plates (Figs 1, 2 and 3).

SUMMARY

The structure of the cuticle of the cysticerci of three species of Taenia was studied by means of optical and electron microscopy. In all three species the cuticle is composed of three layers and covered with hair-like processes. The middle layer, which comprises the bulk of the cuticle, varies in thickness from head to bladder, but there are no differences in thickness between the species studied. The hairs are composed of a core representing an extension of the middle layer and are covered by a continuation of the outer layer. The arrangement of these hairs varies in the species studied.

INTRODUCTION

The cuticle of cysticerci has been described by several investigators (Leuckart, 1886; Young, 1908; Crusz, 1948; Holz and Pezenburg, 1957; and Voge, 1962). Their accounts differ, however, with regard to the number of layers forming the cuticle, the nature of the hair-like processes and the relation of these hairs to the cuticle and subcuticular tissues. Leuckart (1886) referred to earlier descriptions of superficial hairs in Cestodes, but attached no importance to them. Young (1908) noted the hairs on *Cysticercus pisiformis* and suggested that they were extensions of

parenchyma fibres, while Holz and Pezenburg (1957) thought that the hairs in Cysticercus bovis originated from minute granules situated beneath the cuticle, although such a connection was not actually demonstrated. The present study utilising the electron- as well as the optical- microscope was undertaken with the aim of resolving the differences appearing in extant descriptions.

MATERIALS AND METHODS

The whole of the material used of Cysticercus bovis (Taenia saginata) was obtained from a single bullock, slaughtered in the Edinburgh abattoir, which had a severe generalised infestation. All the cysts used were taken from the masseter muscle from which 49 cysts were recovered altogether. Cysts were also collected from other body sites but these were not utilised for the present purpose.

Cysticercus tenuicollis (Taenia hydatigena), which is more abundantly available from abattoir sheep, was collected from the mesenteries on several occasions.

Cysticercus pisiformis was recovered from the mesenteries of laboratory maintained rabbits 8 weeks after ingestion of Taenia pisiformis eggs collected from an experimentally infested dog.

For optical microscopy the cysticerci were always freed from their capsules before fixation. The following fixatives were used:- Helly, Bouin, Carnoy and Fleming fluids (Baker, 1945, Pantin, 1948). The period of fixation for the first two fixatives was six hours and for the last two it was two hours. After thorough washing and removal of excess fixative the cysticerci were dehydrated to 96% ethanol and transferred to methylbenzoate and 96% ethanol (1:1). They were then passed through methylbenzoate to pure benzene before

embedding in paraffin wax. Sections were cut with the microtome set at 5 and stained variously with Ehrlich's haematoxylin and eosine, Heidenhain's iron haematoxylin and Mallory's triple stain (G.T. Gurr).

For electron microscopy the cysts were opened and small pieces of tissue of approximately 2 mm. square were excised and fixed in 1% osmium tetroxide and buffered at pH 7.5 with veronal acetate (Palade, 1952). After dehydration in ethanol and infiltration in N-butylmethacrylate they were embedded in a mixture of 93 parts N-butylmethacrylate and 7 parts methylmethacrylate to which 1% (V/V) benzoyl-peroxide had been added as catalyst. Tissues were sectioned at approximately 250Å using a Huxley microtome and examined with a Siemens Elmiskop I electron microscope. Micrographs were taken as required.

RESULTS

Cysticercus bovis. Within the limits studied the thickness of the cuticle appears to be independent of the size of the cyst, although the present material ranged from 4 mm. to 7 mm. in major diameter. This variation in size largely reflects differences in the stage of development reached, since the larger cysts possessed more completely developed suckers than the smaller ones. Within the individual there is marked variation in cuticle thickness, ranging from 5 over the invaginated head down to 1 over the bladder with a gradual transition between. Three distinct layers are readily distinguished in the cuticle of sections stained with Mallory's triple stain, which colours the external and basal layers blue, and the middle layer red.

The external layer is very thin and was found to be loose or absent over the head in larger cysts. The middle layer is homogene in appearance and forms the bulk of the cuticle. It is this layer which varies markedly in thickness and so produces the differences described in total cuticle thickness. Beneath this layer there is basal layer of thin fibres which is always distinct. The two haematoxylin preparations stain the cuticle uniformly without distinction between the three layers.

The surface of the cyst in this species is densely covered by fine hairs which vary in length from about 2 to 4 They are longer on those parts of the cuticle which have the middle layer thicker except on the invaginated cuticle of the head where they are always lacking (Fig. 1A and B). The structure of these hairs is no discernible with the optical microscope on account of their poor affinity for stains.

Under the electron microscope the middle layer is found again to be the major component lying between very thin external and basal layers. The structure of the superficial hairs is now manifest. The middle layer is drawn out into fine villus-like processes over which the external layer is continuous. Thus the hairs are made up of two of the described cuticular layers (Fig. 1E).

C. tenuicollis. The structure of the cuticle of this species is essentially similar to that of C. bovis. The three layers, the variation in thickness and the superficial hairs are all shown in Fig. 2, A to E. The hairs which appear to be somewhat longer are sparser and more widely spaced than are those of C. bovis.

C. pisiformis. The main feature of this species is the discontinuous distribution of the superficial hairs. These are

much sparser than in the two previous species and are confined to irregularly scattered groups. They are, moreover, much shorter tha in the other two species. Whereas the cuticle in C. bovis and C. tenuicollis is quite smooth in appearance, that of C. pisiformis thrown into folds in places and over these the hairs are absent.

It is remarkable that the thickness of the cuticle is of the sam order for all three species described, although the cysts themselves differ greatly in size. Thus, in C. bovis of about 5 mm. length, t cuticle is of about the same thickness as in C. pisiformis of some 1 to 12 mm. or C. tenuicollis measuring 40 mm. or more.

DISCUSSION

Young (1908) in his studies on the histogenesis of C. pisiformis recognised two cuticular layers of which the outermost was sometimes missing. His use of haematoxylin dyes, however, which we find to stain all cuticular components uniformly, could account for his failure to recognise all three layers of the cuticle. Crusz (1948) and Voge (1962) both describe the cuticle of C. tenuicollis as a single-layered structure, which stains blue with Mallory's triple stain. This result is quite different from the present findings in which the predominant colour (middle layer) with Mallory's stain is red. This difference must be attributed to differences in technique since, according to Pantin (1948), differences in timing and procedure produce considerable variation in result with different tissues and different brands of stain. Voge (1962) identified a thin blue layer beneath the cuticle and this may correspond to the basal cuticular layer of the present work. On the other hand, Holz and Pezenburg (1957) positively deny the

existence of a basement membrane beneath the cuticle and describe a continuity between cuticle and underlying cells mediated by fine intercommunicating cellular processes. Such structures were never observed in the present studies, in which the triple layer structure of the cuticle observed with the light microscope was confirmed by electron-microscopy. Holz and Pezenburg attempt, furthermore, to relate the superficial hairs to the fine subcuticular filamentous processes they describe. Young (1908) also thought that the hairs provided a means of direct communication between the subcuticular tissues and the exterior. We find, on the other hand, that the precise structure and relations of the hairs are impossible of resolution with the light microscope. Electron-microscopy shows quite clearly that the hairs are derivatives of the middle cuticular layer covered by the continuous external layer, but there is no evidence for the continuity with subcuticular structures either described or postulated by earlier workers.

It is interesting to note that Ludvik (1960) demonstrated simil hair-like processes on the cyst wall of Sarcocystis miescheriana by electron-microscopy, and suggested for them a possible nutritional role by increasing the surface area of the parasite. If a similar role be postulated for the hairs of cysticerci it perhaps becomes significant that in the species where hairs are sparsest, namely, C. pisiformis, the cuticular surface is increased by a distinct rugosity.

ACKNOWLEDGMENTS

This work was financially supported by the Colombo Plan authorities in the United Kingdom to whom I am greatly indebted. I thank also the members of the staff of the Parasitology Sub-Department at Edinburgh, and Dr. J.R. Baker, F.R.S., of the Department of Zoology, Oxford, for their guidance and encouragement. Finally, I am most grateful to Dr. D.C. Barker and Mr. A.E.G. Dunn for carrying out the electron microscope procedures.

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ABBREVIATIONS

used in figures 1-79 are as follows:-

A - Cuticle
B - Scolex or head
BI - Neck
C - Bladder
D - Cavity
E - Strobila
F - Suckers
FI - Hooks
G - Parenchyma
H - Muscle fibres
I - Hairs
J - Cortical region
K - Medullary region
L - Nuclei
M - Scolex canal
N - External layer
O - Middle layer
P - Basal layer
Q - Vacuoles
R - Mitochondria-like bodies
S - Fibres
T - PAS positive granules
U - Sudanophilic globules
V - Phospholipid globules
W - Calcium
X - Central vacuole
Y - Wall of cyst
Z - Macrophages
ZI - Host capsule
ZII - Excretory canal

FIGURE 1.

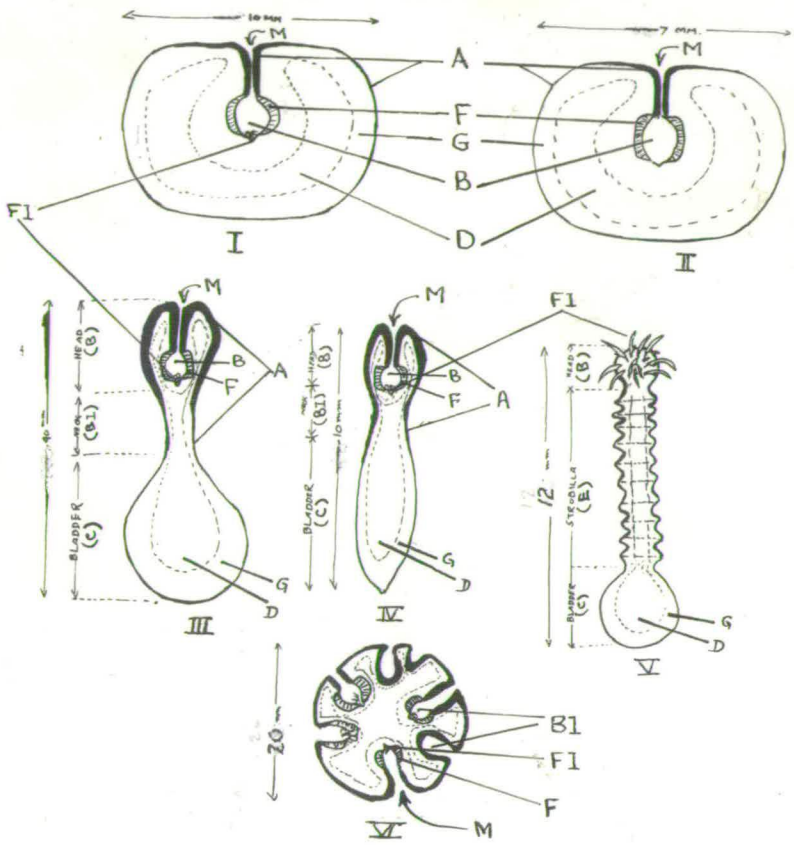

Fig.1. Diagram showing morphology of fully developed cysticerci:
(i) Cysticercus cellosae
(ii) Cysticercus bovis
(iii) Cysticercus tenuicollis
(iv) Cysticercus pisiformis
(v) Cysticercus fasciolaris
(vi) Coenurus cerebralis

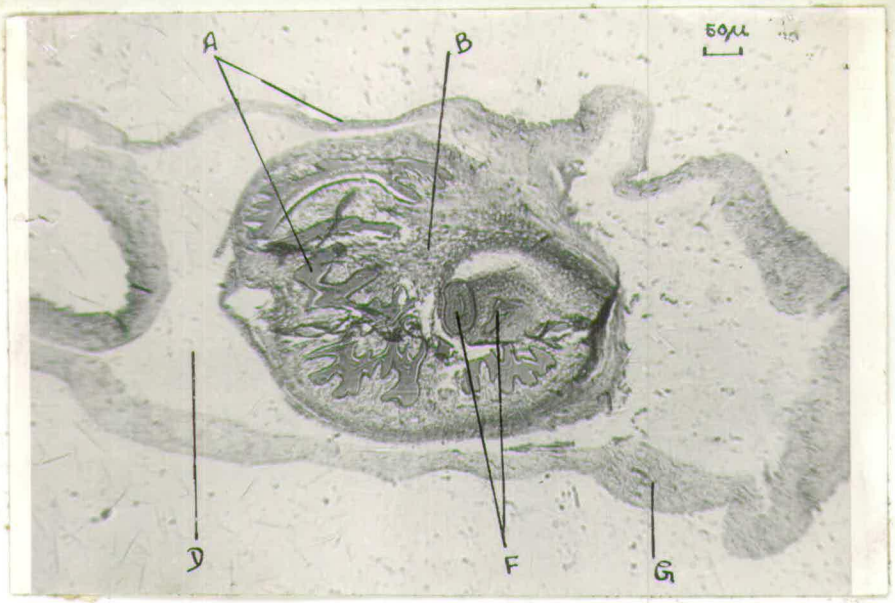

FIG 2

Fig.2. C. bovis. Section of whole cyst. Helly-Mallory (x 2/3). Note: Head occupies a large proportion of central cavity. Thickness of cuticle varies over head and bladder.

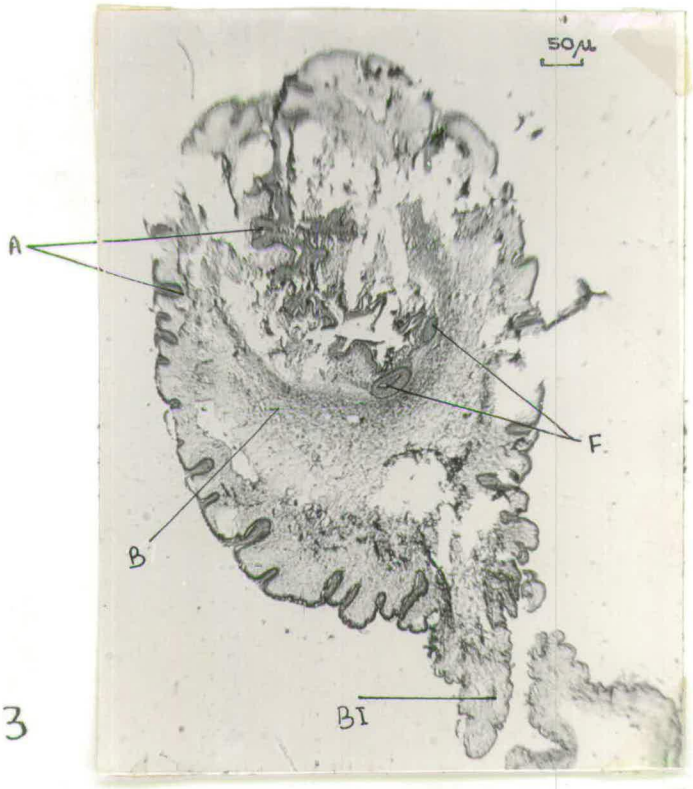

FIG 3

Fig.3. C.tenuicollis. Section of head. Helly-Mallory (x 2/3). Showing invaginated head confined to anterior extremity.

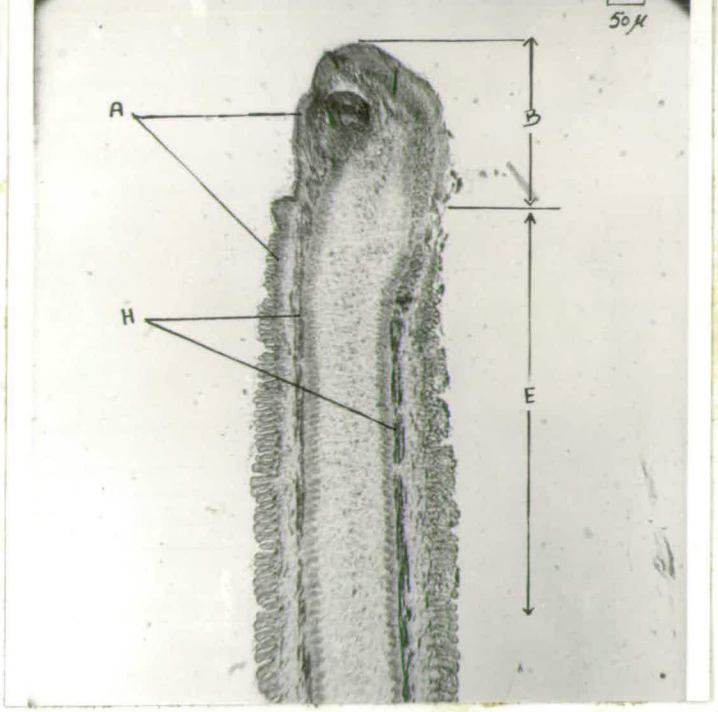

FIG 4

Fig.4. C. fasciolaris. Section of strobilocercus at 60 days. Helly-Mallory (x 2/3). Evaginated head (B) and strobila (E) posterior to head.

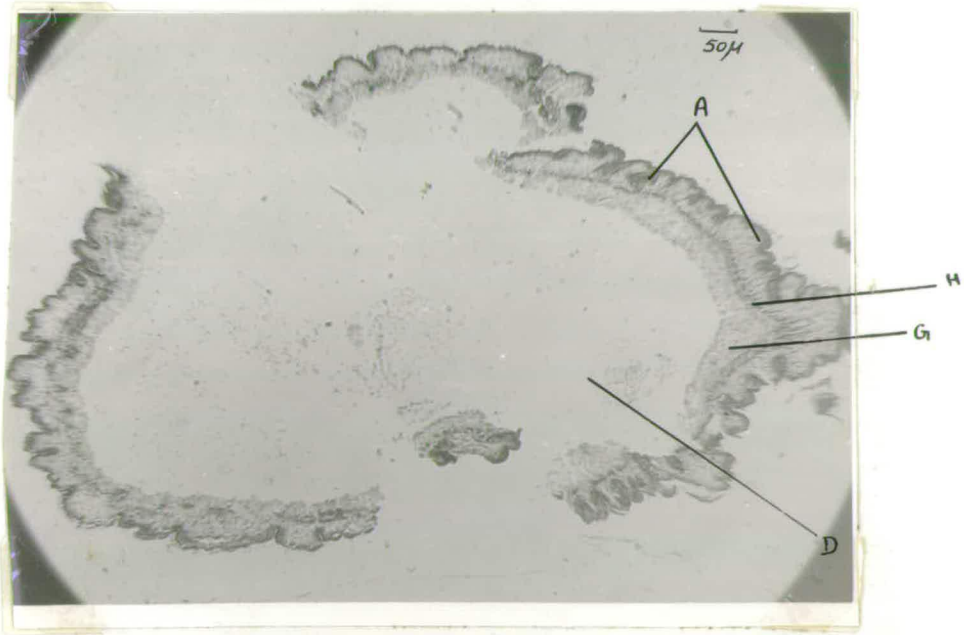

FIG 5

Fig.5. C. fasciolaris. Section of bladder at 60 days. Helly-Mallory (x 2/3).

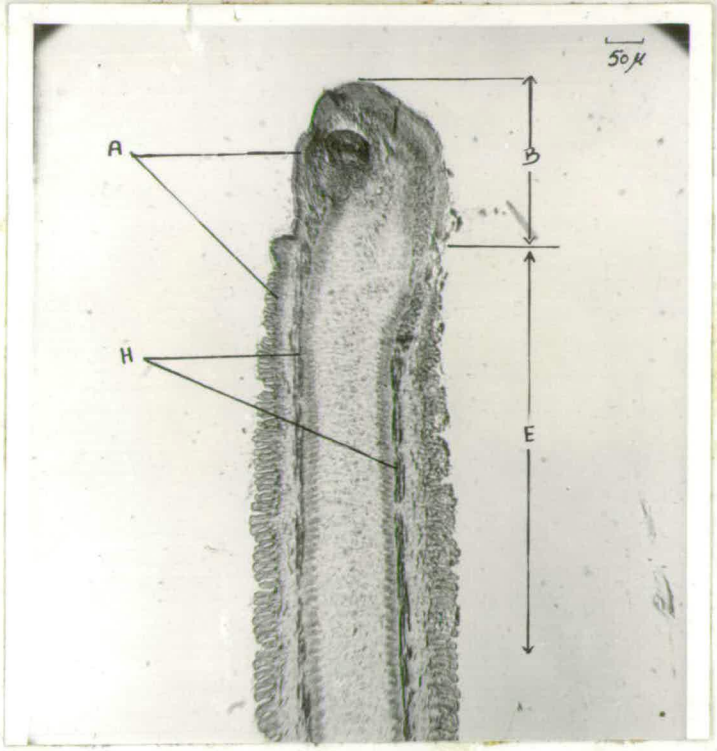

FIG 4

Fig.4. C. fasciolaris. Section of strobilocercus at 60 days. Helly-Mallory (x 2/3). Evaginated head (B) and strobila (E) posterior to head.

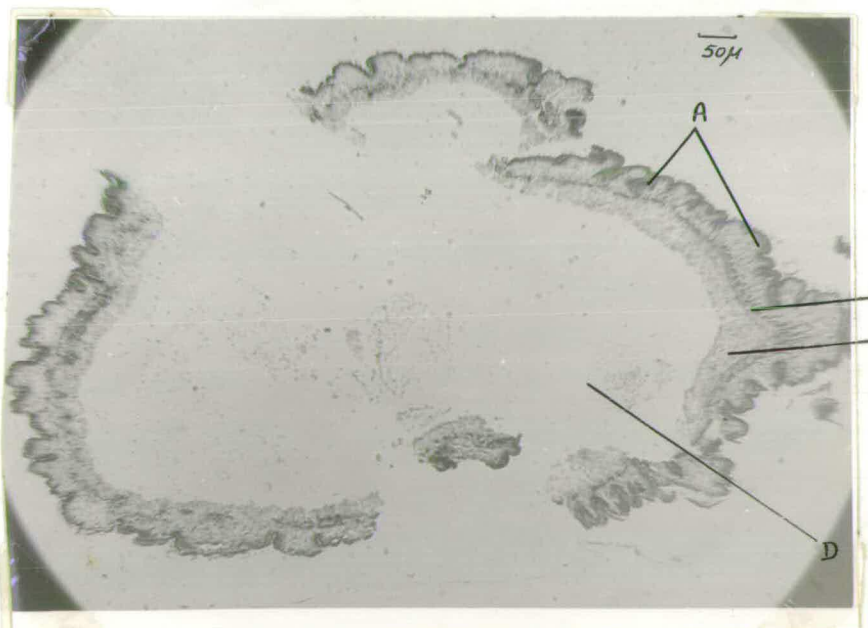

FIG 5

Fig.5. C. fasciolaris. Section of bladder at 60 days. Helly-Mallory (x 2/3).

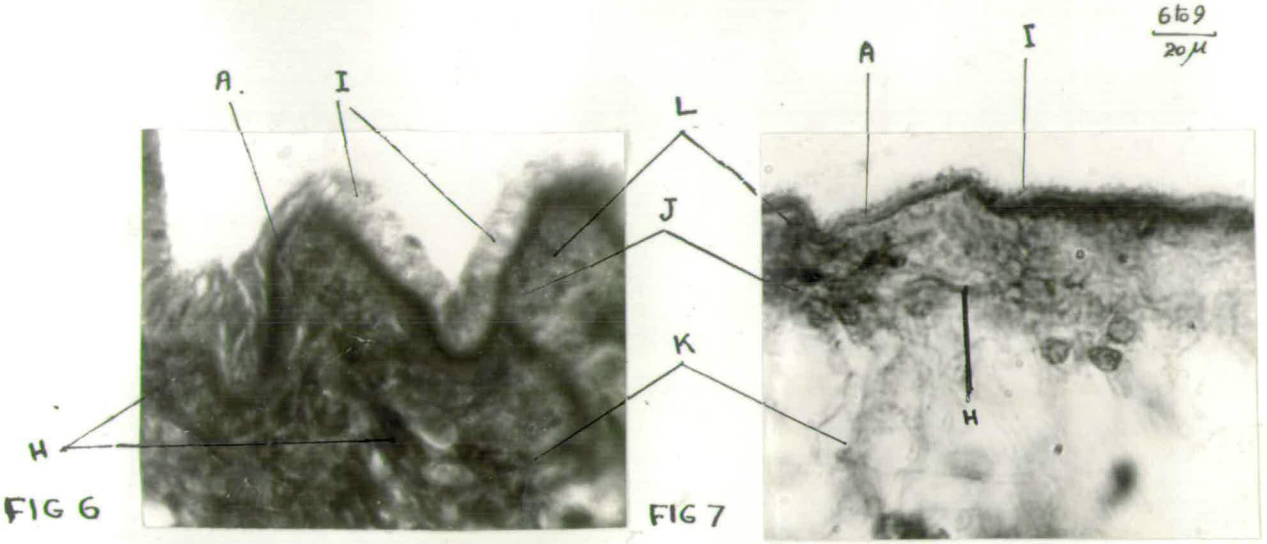

FIG 6 FIG 7

Figs.6 & 7. C. bovis. Sections of bladder of different regions. Helly-Mallory (x 1/6 dry).
Note. Length of hairs (i) and compare thickness of cuticle (A) in the two figures.

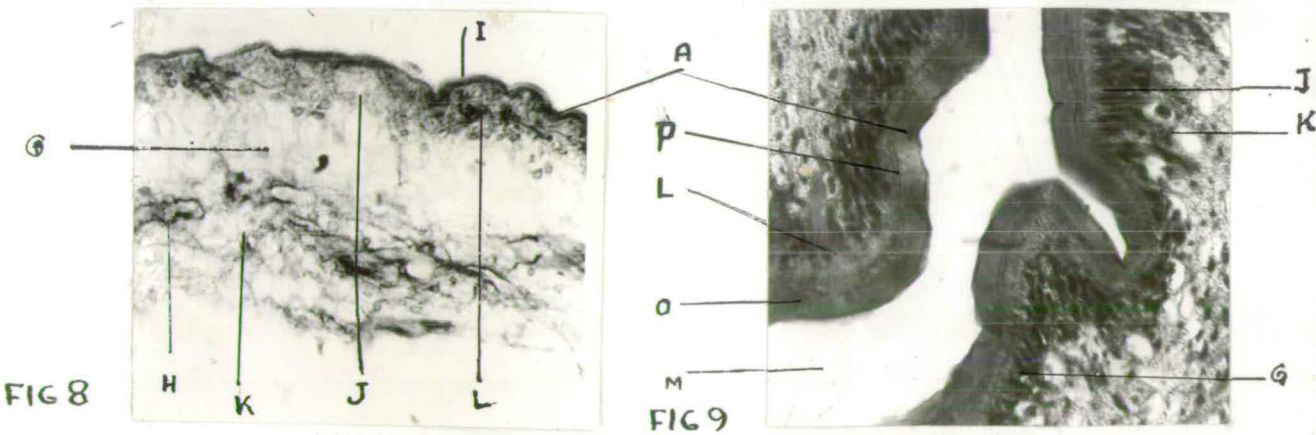

FIG 8 FIG 9

Figs.8 & 9. C. bovis. Sections of bladder and head region. Helly-Mallory (x 1/6 dry).
Note. Absence of hairs in fig.9 head region and compare thickness of cuticle in head and bladder region in the two figures.

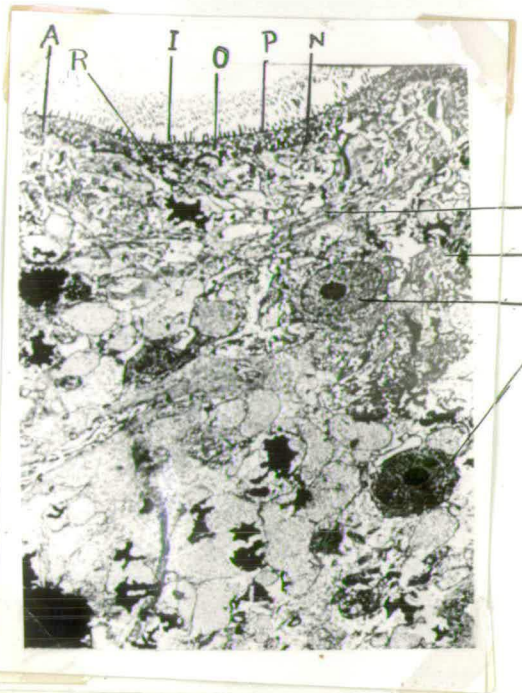

FIG 10

Fig.10. C. bovis. Section of bladder fixed Ismium.
Electron micrograph (x 2000).
Note. Hairs (i) and dark bodies in cuticle (A).

FIG 11

Fig.11. C. bovis. Section of bladder fixed Osmium.
Electron micrograph (x 28,000).
Note. Three layers of the cuticle (A) and
mitochondria-like bodies (R).

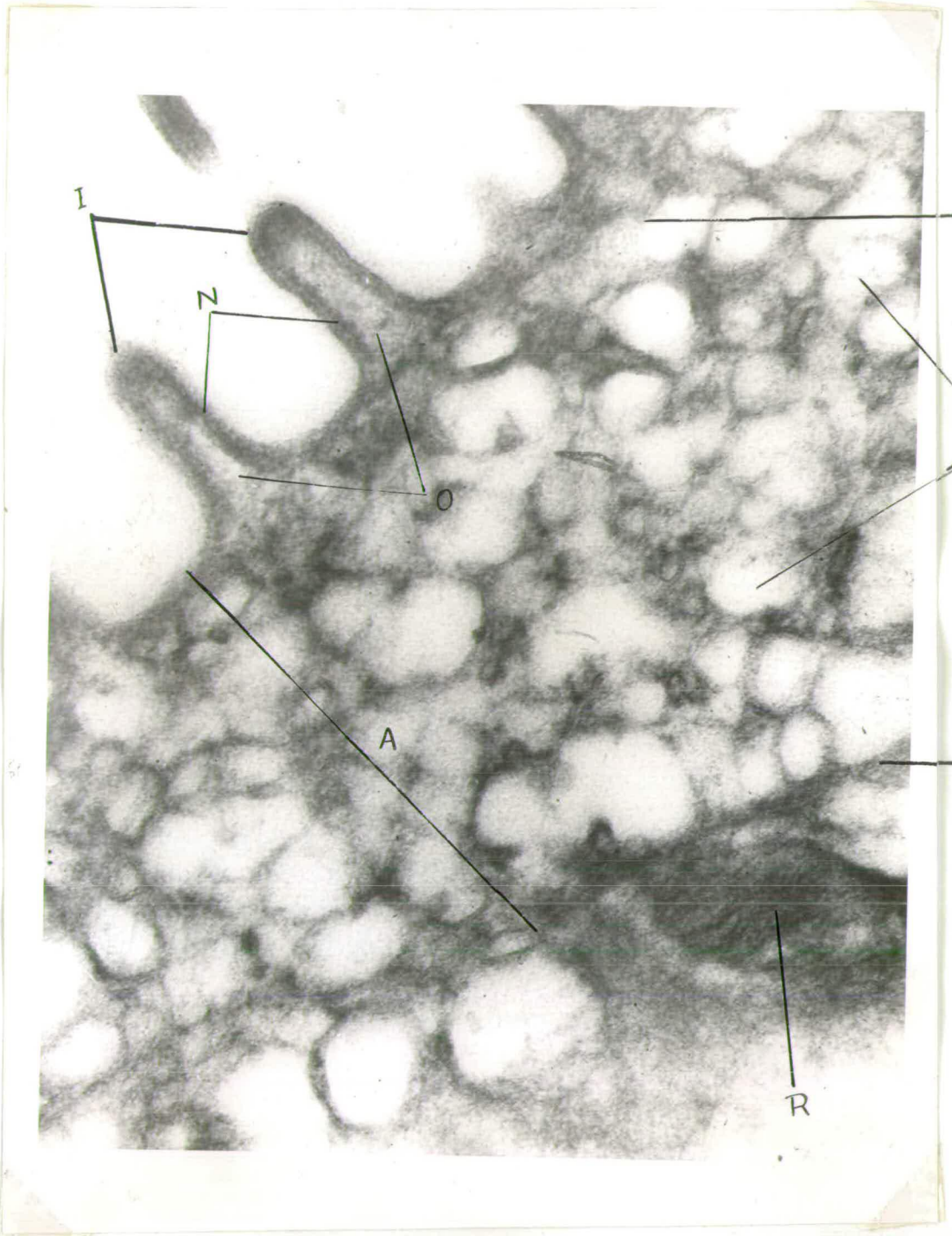

FIG 12

Fig.12. C. bovis. Section of bladder fixed Osmium. Electron micrograph (x 80,000).
Note. Hairs, the villus-like structure composed of external and middle layers of the cuticle (A). In parenchyma (G) mitochondria-like bodies.

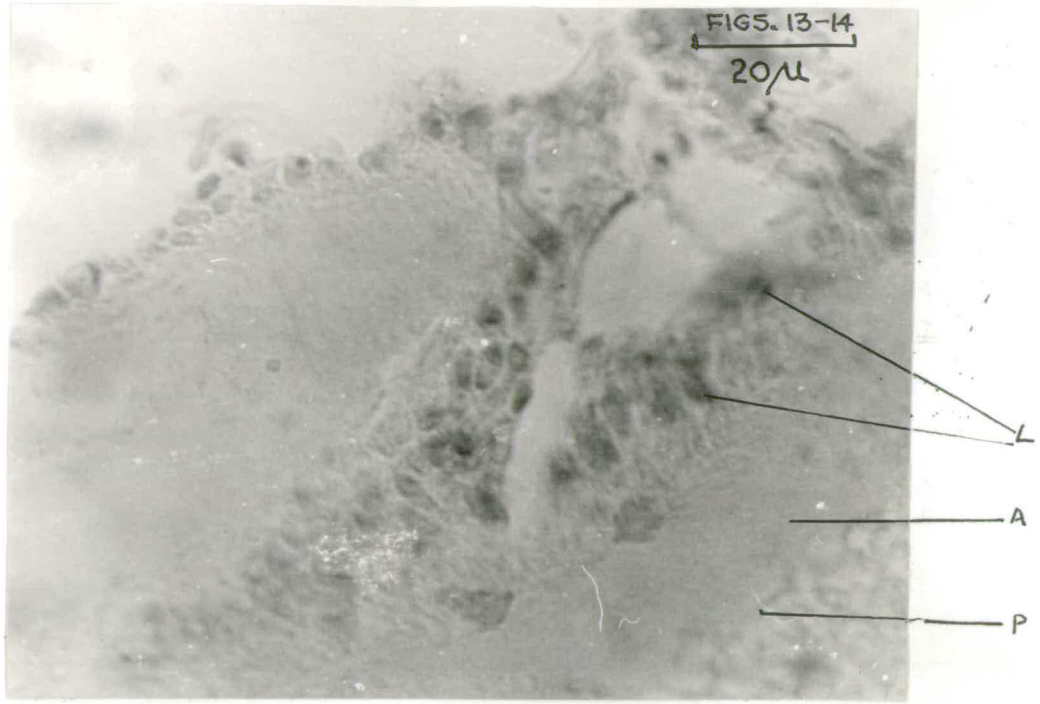

FIG 13

Fig. 13. C. bovis. Section of head. Helly-Haematoxylin and eosin (x 1/12 oil).
Note. Palisade nuclei (L) close to the cuticle (A).

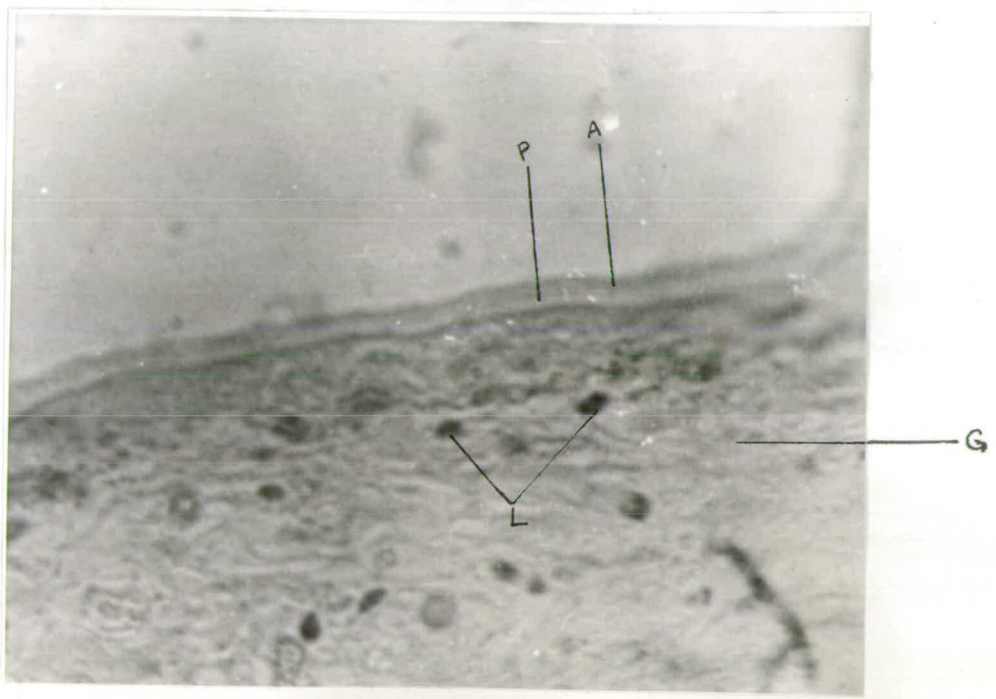

FIG 14

Fig. 14. C. bovis. Section of bladder. Helly-Haematoxylin and eosin (x 1/12 oil).
Note. Nuclei (L) in parenchyma (G) away from cuticle (A).

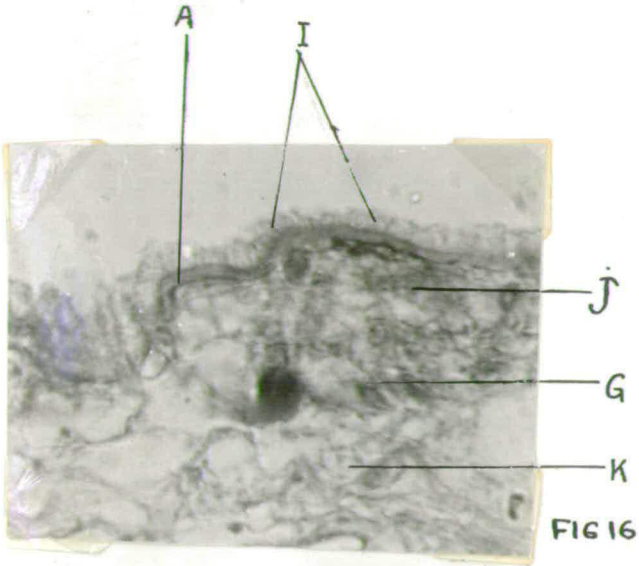

FIG 15

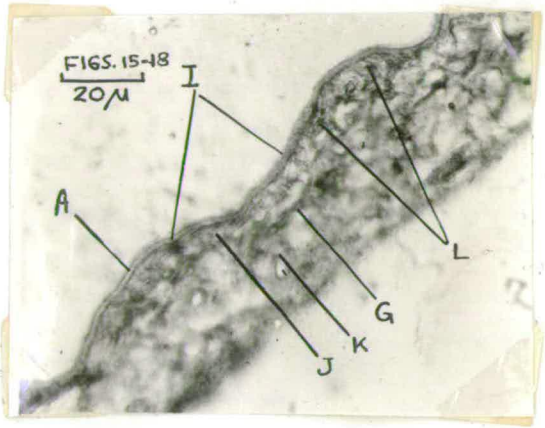

FIG 16

Figs. 15 & 16. C. tenuicollis. Section of bladder of different regions. Helly-Mallory (x 1/6 dry).
Note. Length of hairs (i) and compare cuticle (A) thickness in the two figures.

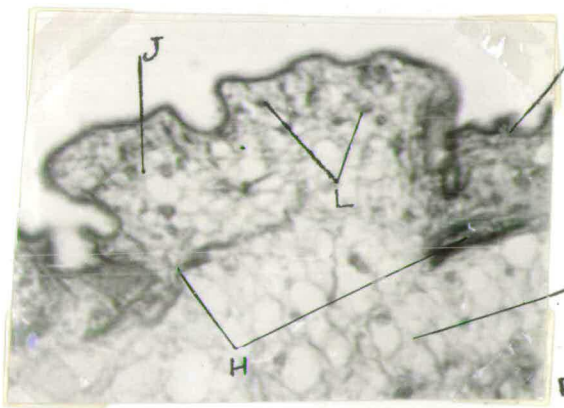

FIG 17

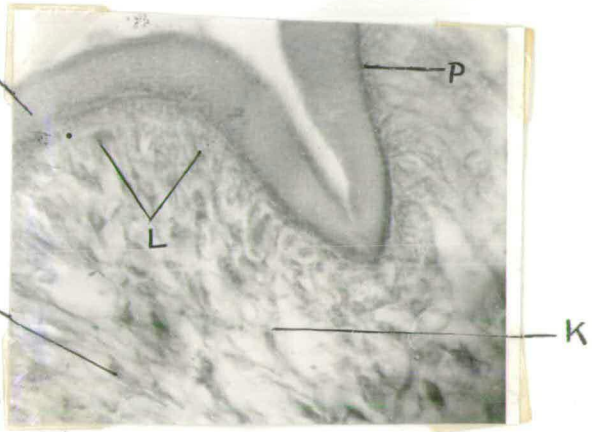

FIG18

Figs. 17 & 18. C. tenuicollis. Sections of bladder and head region. Helly-Mallory (x 1/6 dry).
Note. Absence of hairs and thickness of the cuticle in head region (Fig.18). Compare with bladder region (Fig.17).

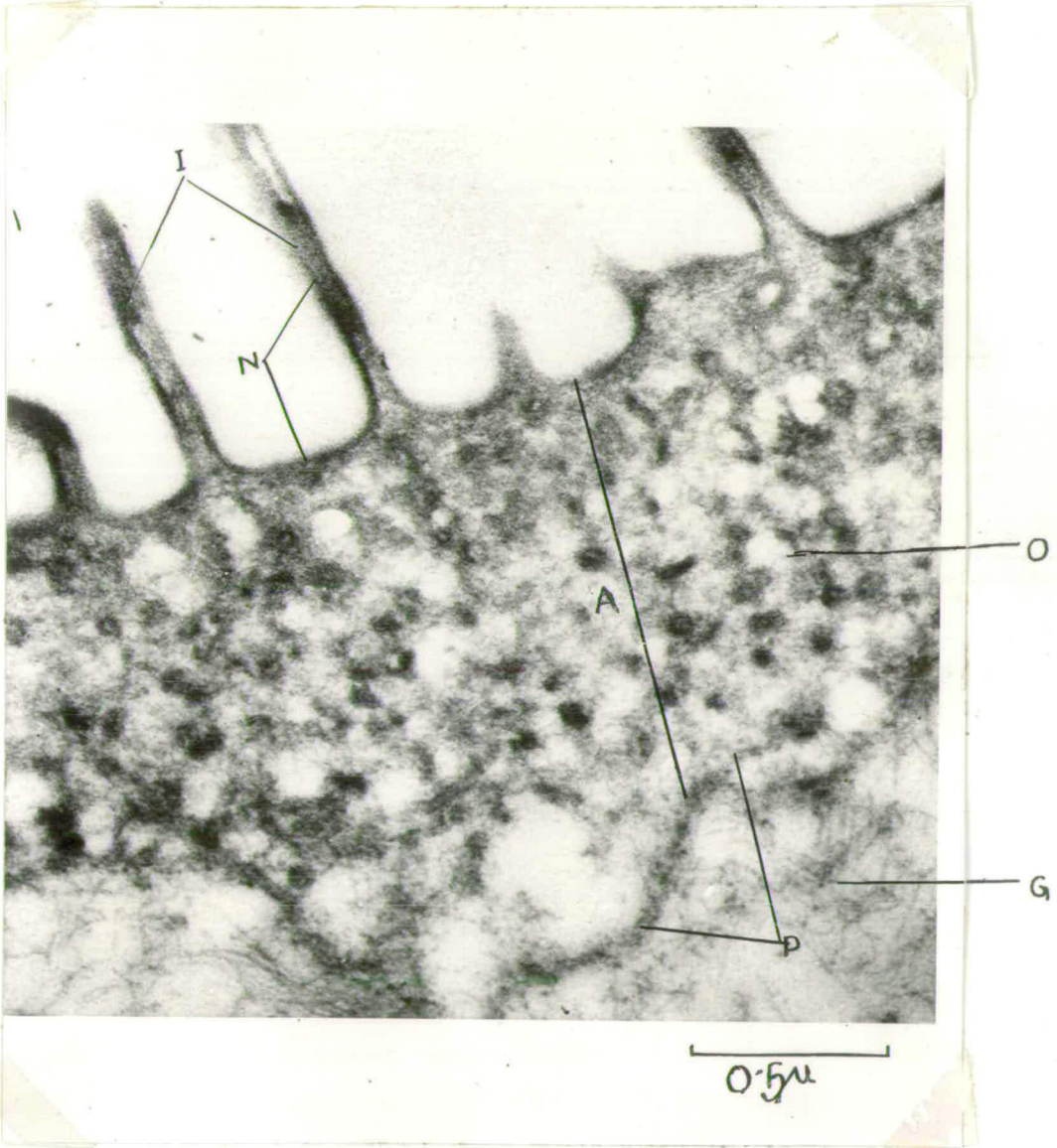

FIG 19

Fig. 19. C. tenuicollis. Section of bladder fixed Osmium. Electron micrograph (x 28,000).
Note. Three layers of cuticle (A). Compare hairs (i) with Fig.11 of C. bovis.

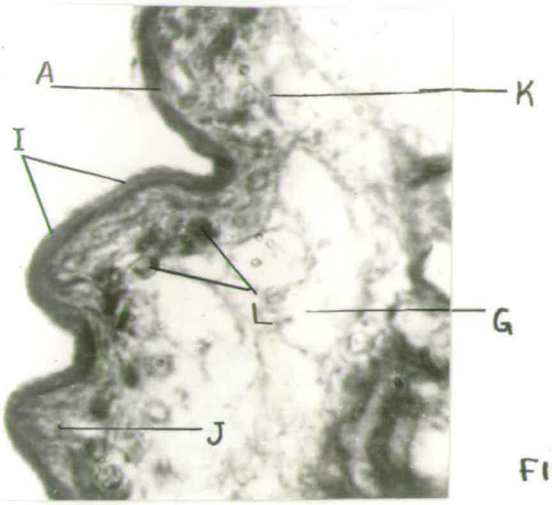

FIG 20

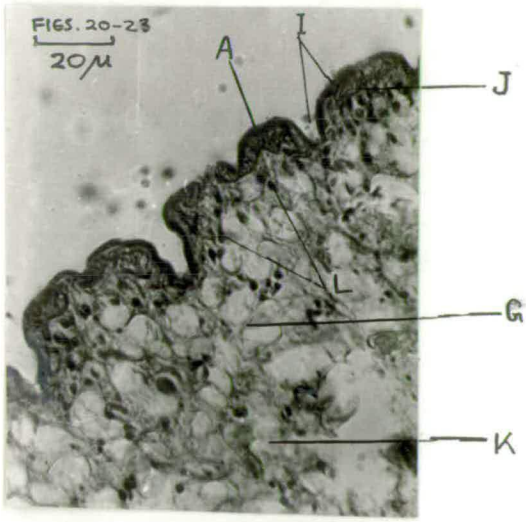

FIG 21

Figs. 20 & 21. C. pisiformis. Sections of different regions of bladder. Helly-Mallory (x 1/6 dry).
Note. Length of hairs (i) and compare cuticle (A) thickness in the two figures.

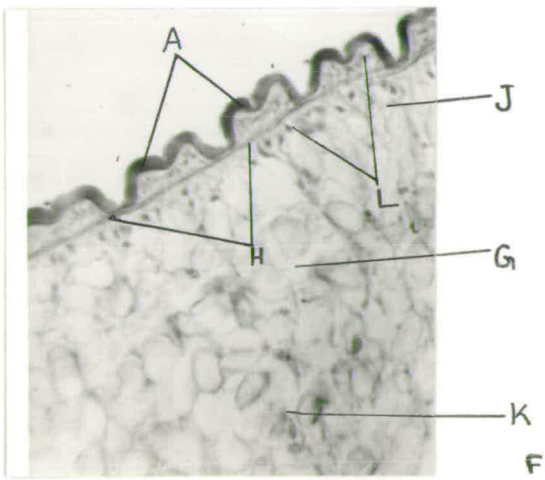

FIG 22

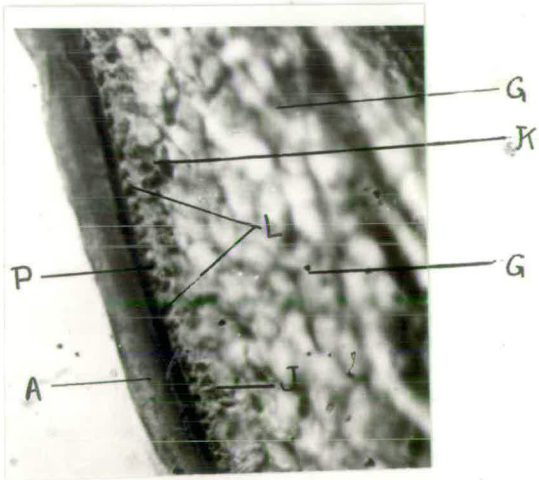

FIG 23

Figs. 22 & 23. C. pisiformis. Sections of bladder and head region. Helly-Mallory (x 1/6 dry).
Note. Absence of hairs and thickness of cuticle in head region (Fig.23). Compare with bladder region (Fig.22).

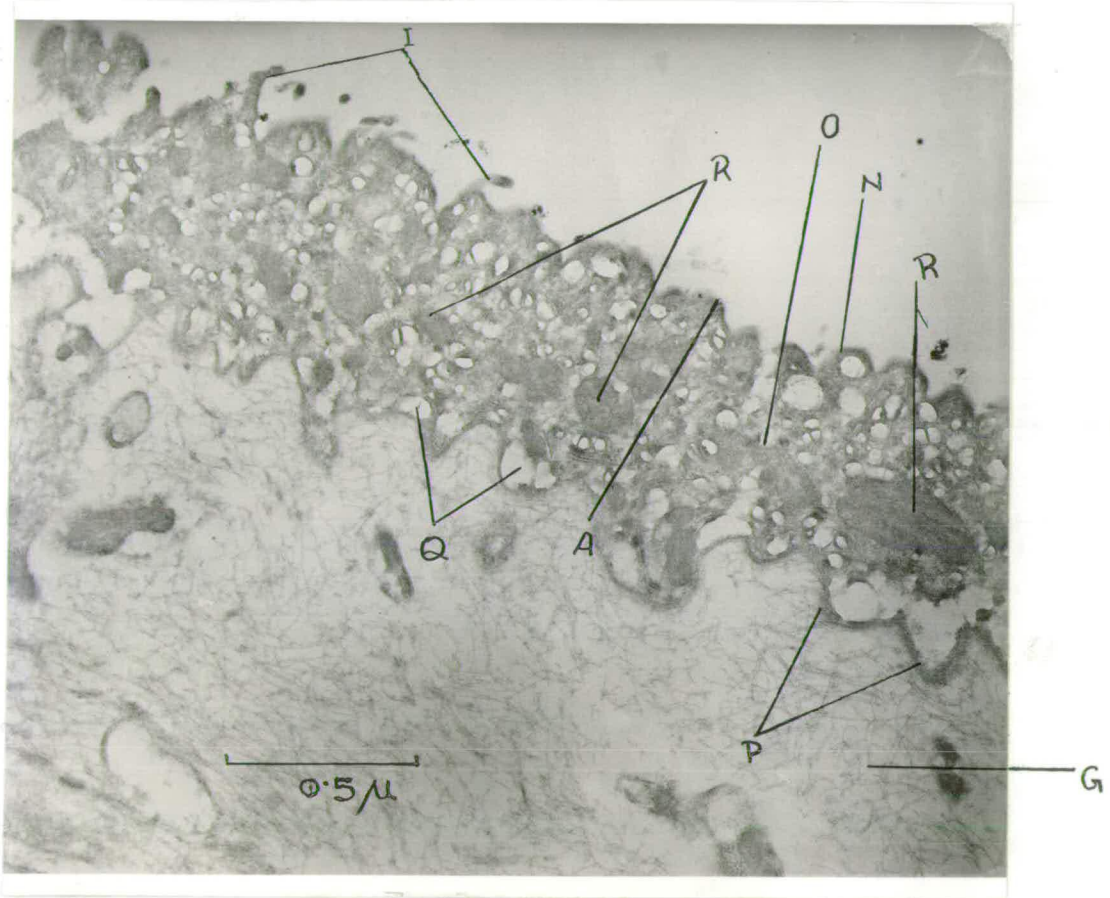

FIG 24

Fig.24. C. pisiformis. Sections of bladder fixed Osmium. Electron micrograph (x 28,000).
Note. Three layers of cuticle (A) and mitochondria-like bodies (R). Compare with figs. 11 and 19.

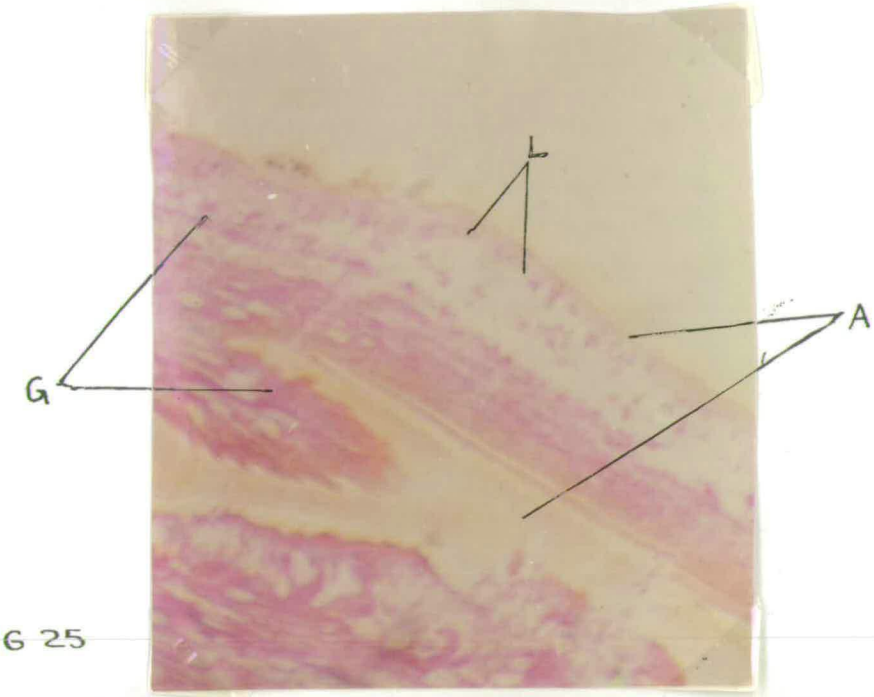

FIG 25

Fig.25. C. bovis. Section of head. Carnoy-Himes triple stain (x 1/6 dry). Showing in cuticle (A) yellow colour = proteins, in parenchyma (G) PA/S positive magenta red colour in granules (T) and in nuclei (L) bluish yellow = DNA.

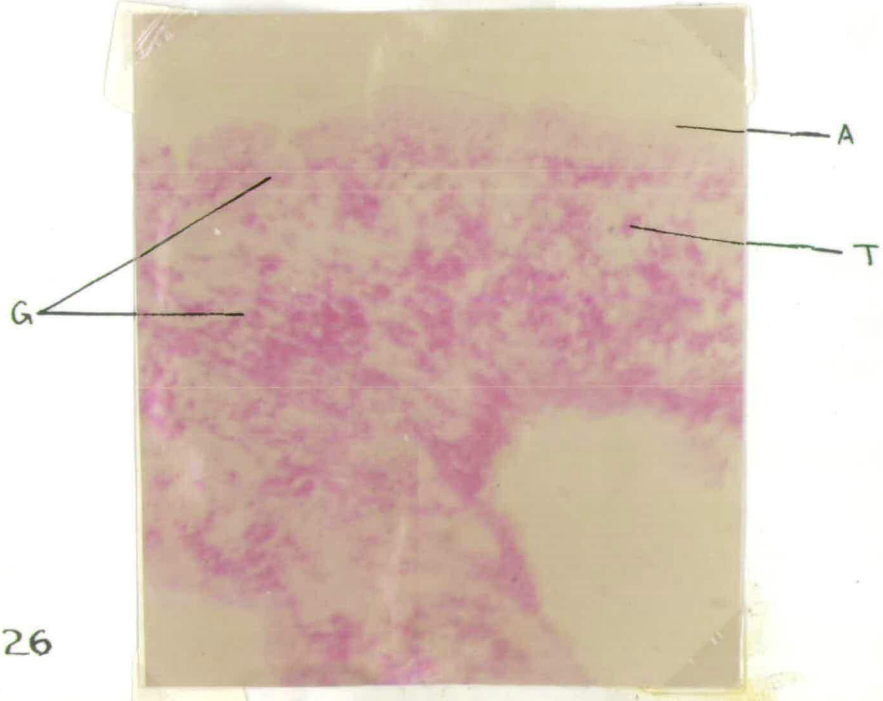

FIG 26

Fig.26. C. bovis. Section of bladder. Carnoy-PA/S (x 1/6 dry). Showing PA/S negative reaction in cuticle (A) and positive reaction in granules (T) in parenchyma (G).

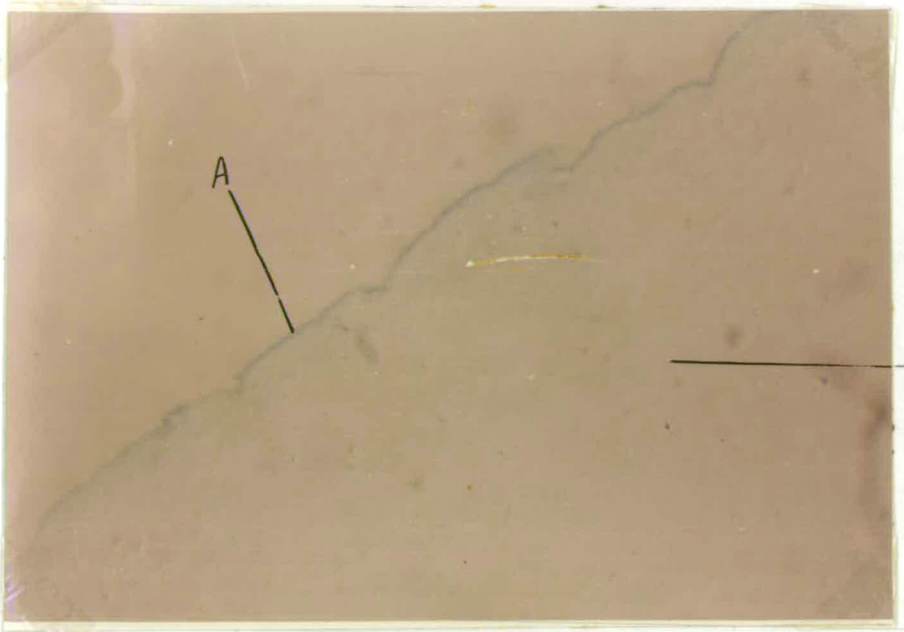

FIG 27

Fig.27. C. bovis. Section of bladder. Carnoy-Alcian blue (x 1/6 dry). Showing bluish green colour in cuticle (A) and very weak blue in parenchyma (G).

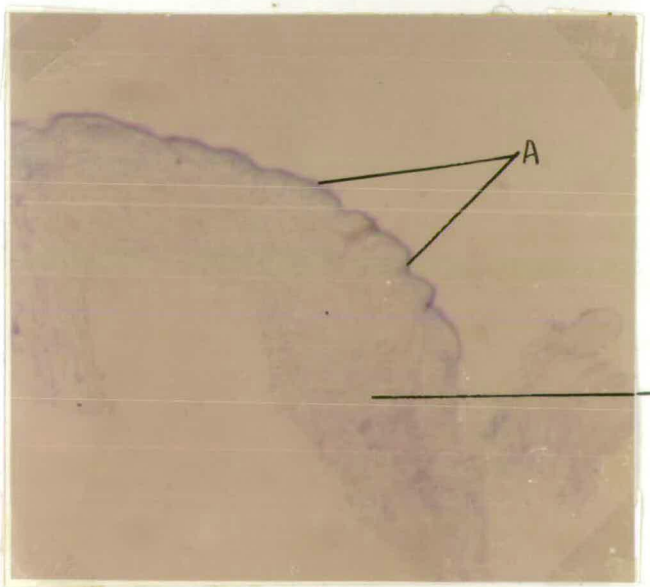

FIG 28

Fig. 28. C. bovis. Section of bladder. Carnoy-Methylene blue extinction test (x 1/6 dry). Showing blue colour at pH 4.1 in cuticle (A) and parenchyma (G).

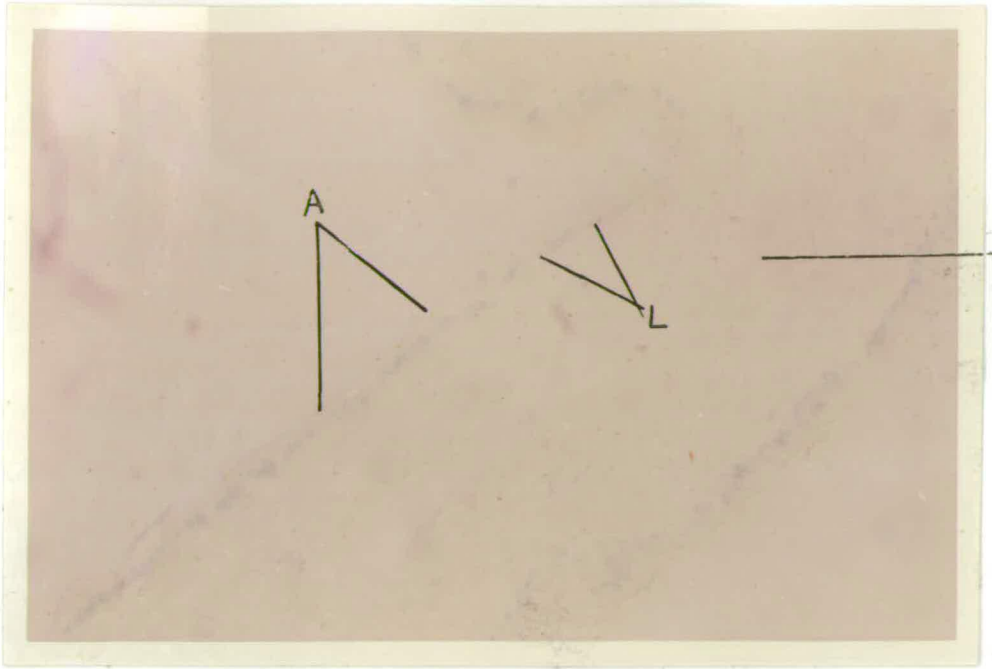

FIG 29

Fig.29. C. bovis. Section of bladder. Carnoy-Methylene blue extinction test (x 1/6 dry). Showing extinction of blue colour at pH 2.2 in cuticle (A) and presence of bluish green colour in nuclei (L) in parenchyma (G).

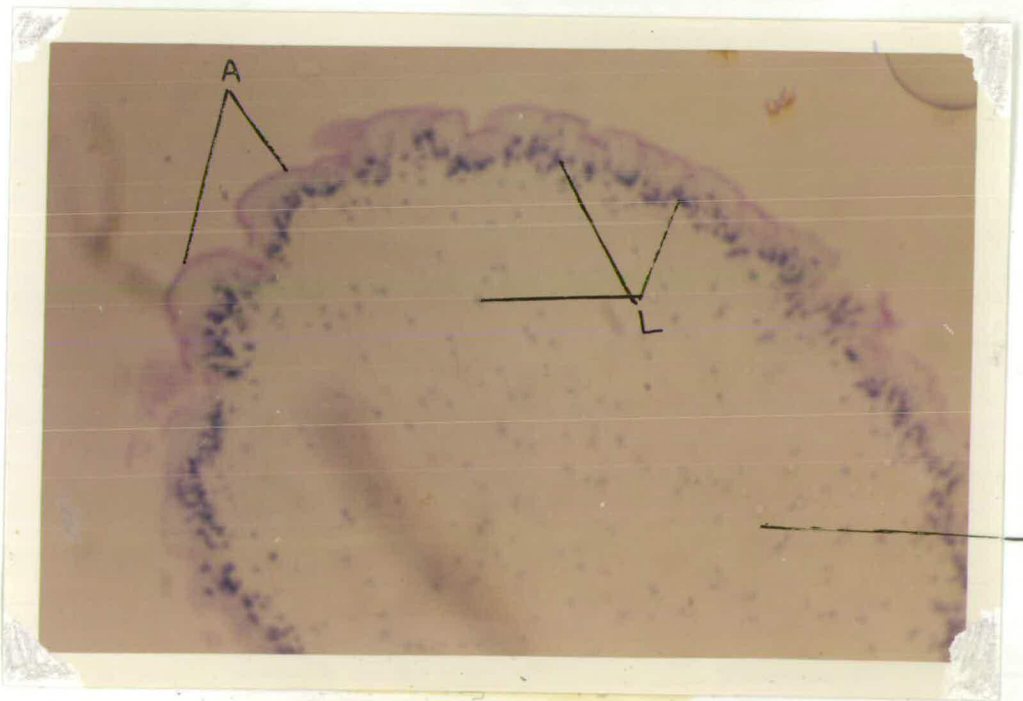

FIG 30

Fig.30. C. bovis. Section of bladder. Carnoy-Toluidine blue (x 1/6 dry). Showing γ metachromasia in cuticle (A) and β metachromasia in nuclei (L).

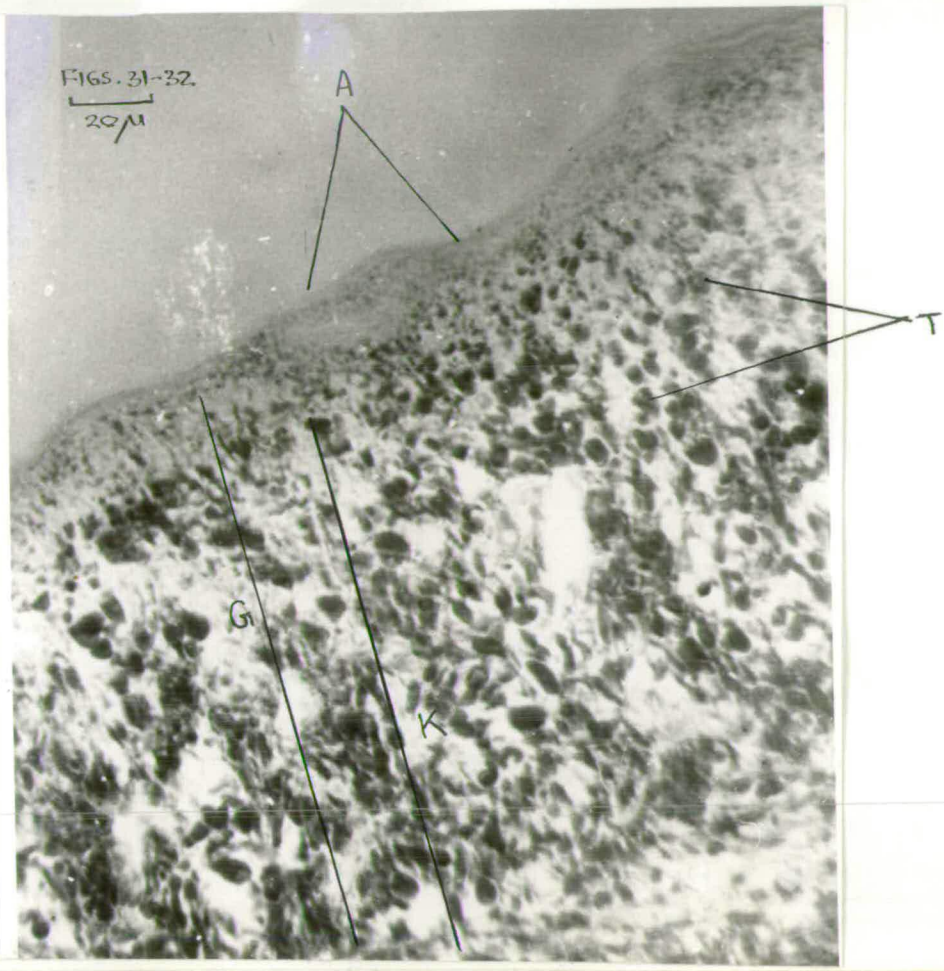

FIG 31

Fig.31. C. bovis. Section of bladder. Carnoy-PA/S (x 1/6 dry). Showing PA/S negative reaction in cuticle (A) and PA/S positive granules (T) in parenchyma (G).
Note. Concentration of granules (T) in medullary region (K).

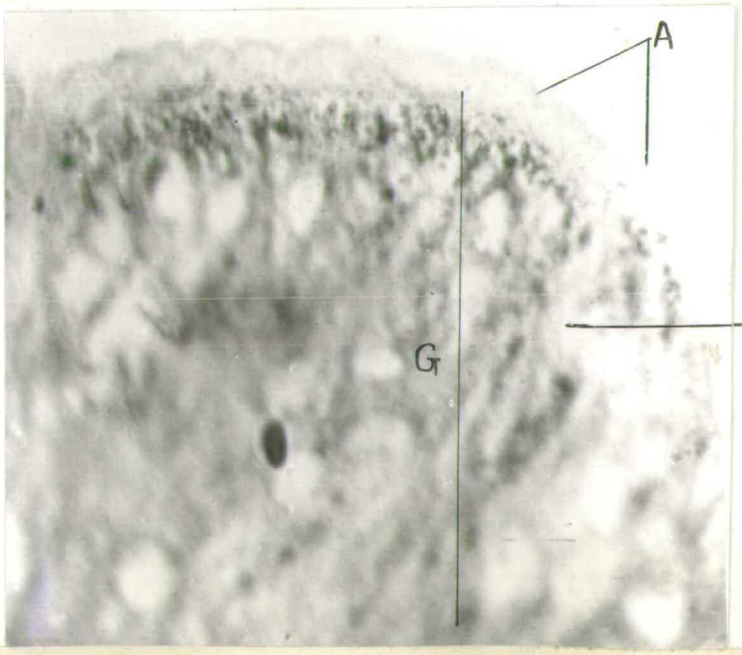

FIG 32

Fig.32. C. bovis. Section of bladder. Carnoy-PA/S after malt diastase (x 1/6 dry) showing absence of PA/S positive granules (T) in parenchyma (G).

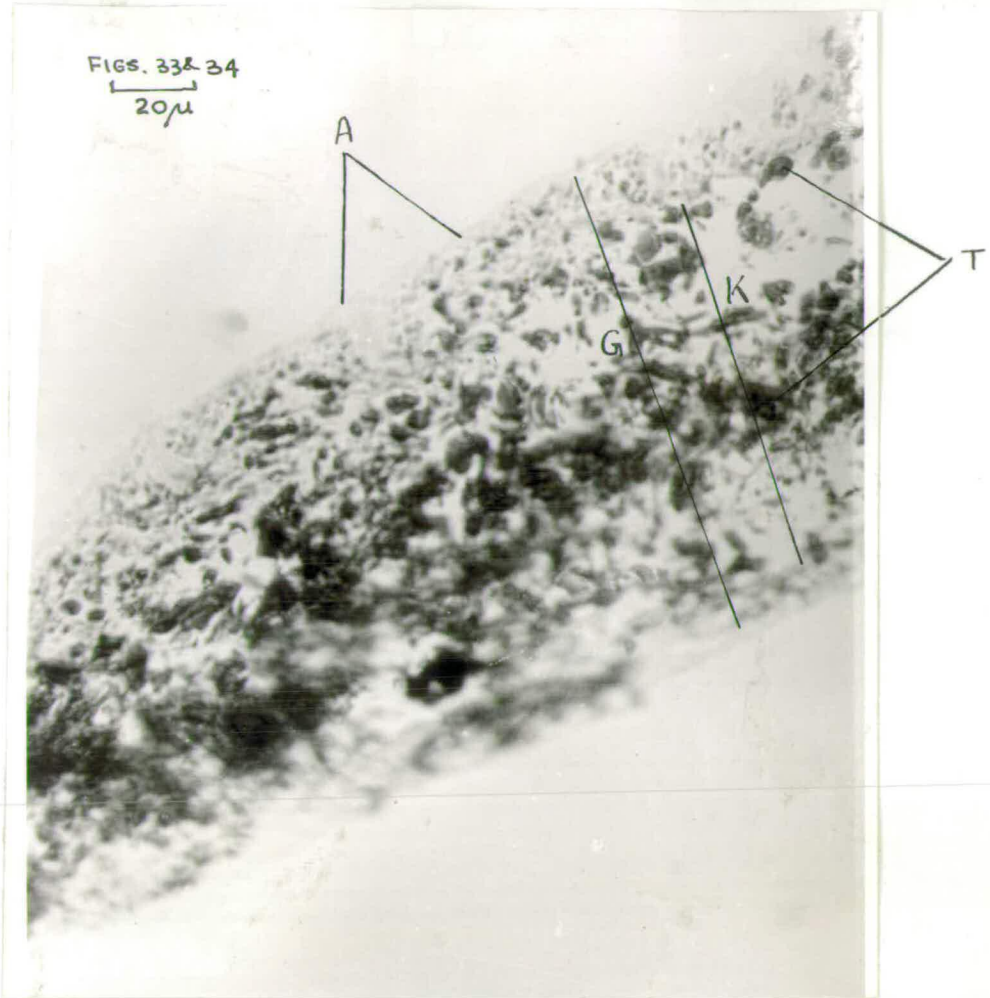

FIG 33

Fig.33. C. tenuicollis. Section of bladder. Carnoy-PA/S (x 1/6 dry). Showing PA/S negative reaction in cuticle (A) and PA/S positive granules (T) in parenchyma (G).
Note. Concentration of granules (T) in medullary region (K).

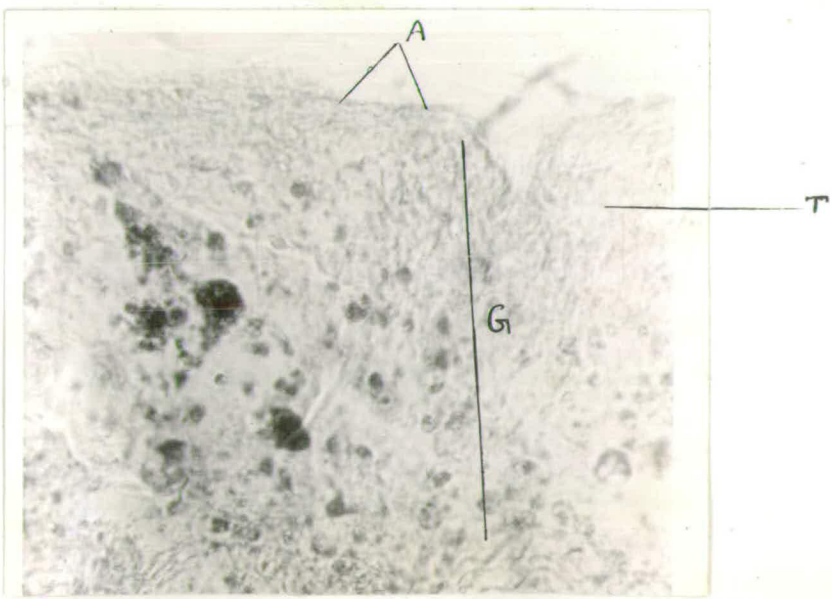

FIG 34

Fig.34. C. tenuicollis. Section of bladder. Carnoy-PA/S after malt diastase (x 1/6 dry). Showing absence of PA/S positive granules (T) in parenchyma (G).

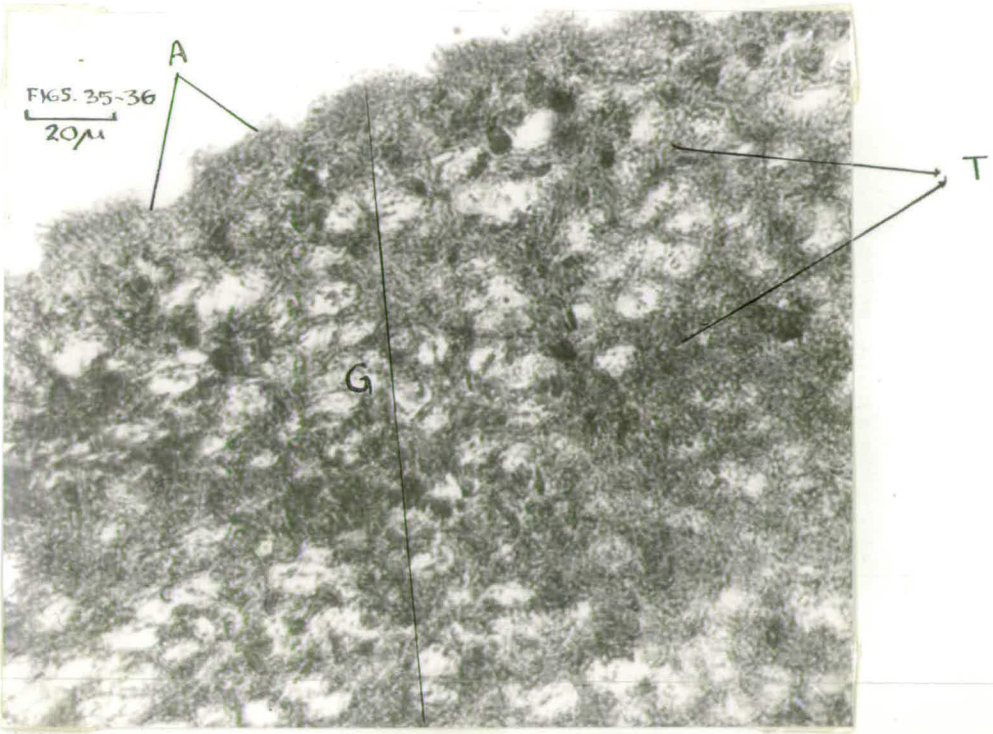

FIG 35

Fig.35. C. bovis. Section of bladder. Zenker-PA/S (x 1/6 dry). Showing PA/S negative reaction in cuticle (A) and PAS positive patches in parenchyma (G).

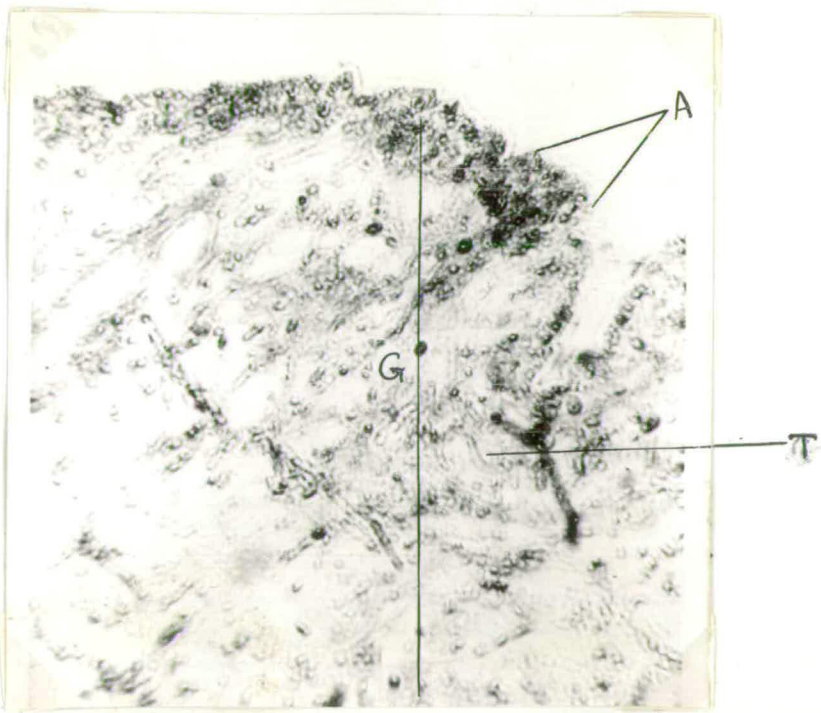

FIG 36

Fig.36. C. bovis. Section of bladder. Zenker-PA/S after malt diastase (x 1/6 dry). Showing absence of PA/S positive patches from parenchyma (G).

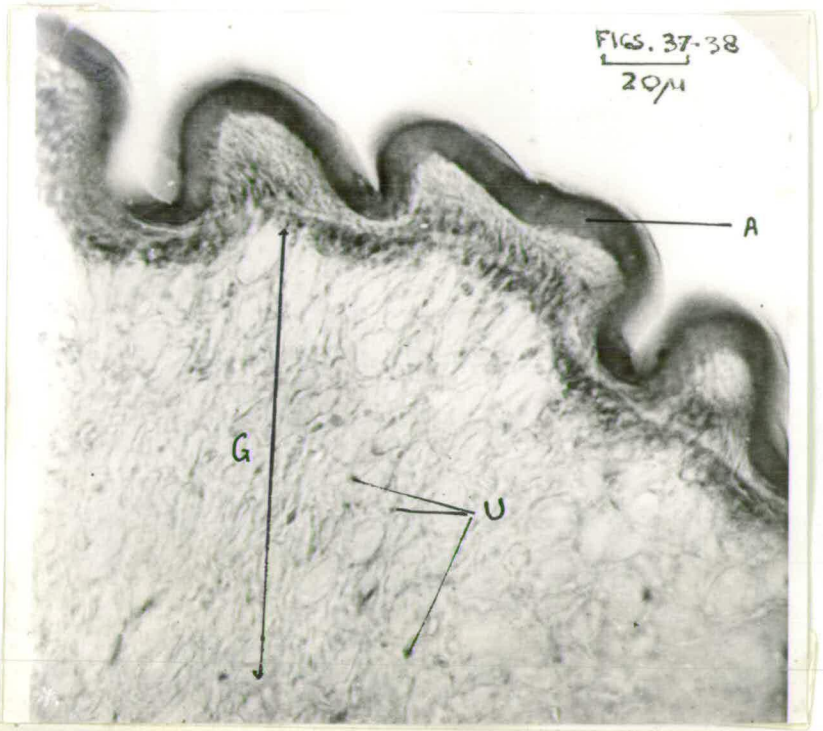

FIG 37

Fig.37. C. bovis. Section of head. Formol calcium frozen section-Sudan Black B (x 1/6 dry). Showing sudanophilia in cuticle (A) and sudanophilic globules (U) spareley distributed in parenchyma (G).

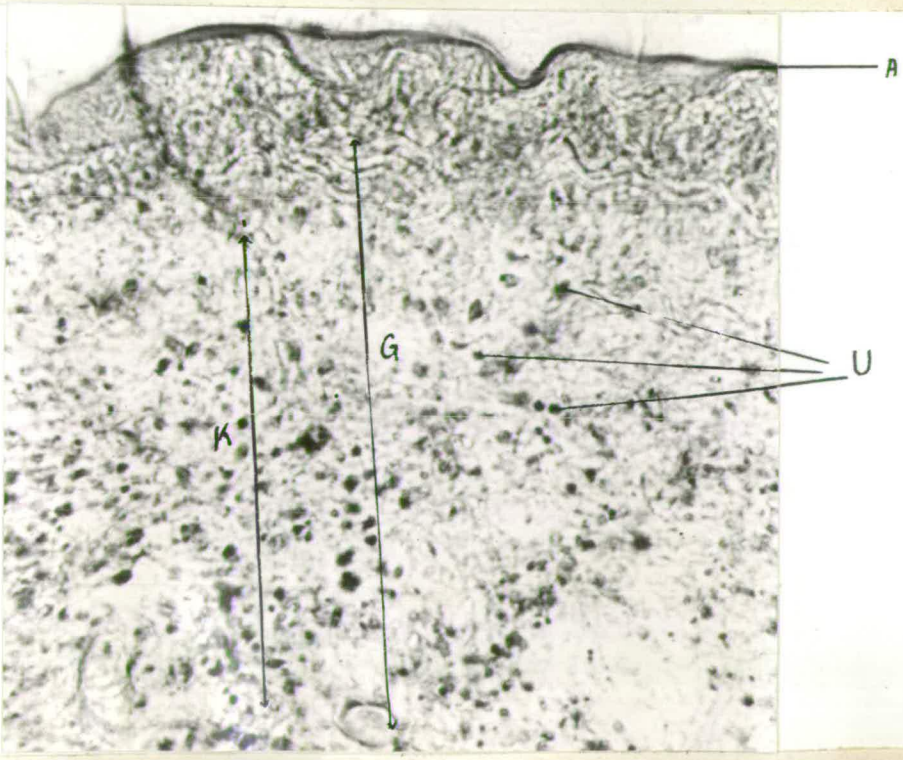

FIG 38

Fig.38. C. bovis. Section of bladder. Formol calcium frozen section-Sudan Black B (x 1/6 dry) showing sudanophilia in cuticle (A) and sudanophilic globules (U) concentrated in parenchyma (G). Note. Compare sudanophilic globules in parenchyma in fig.37.

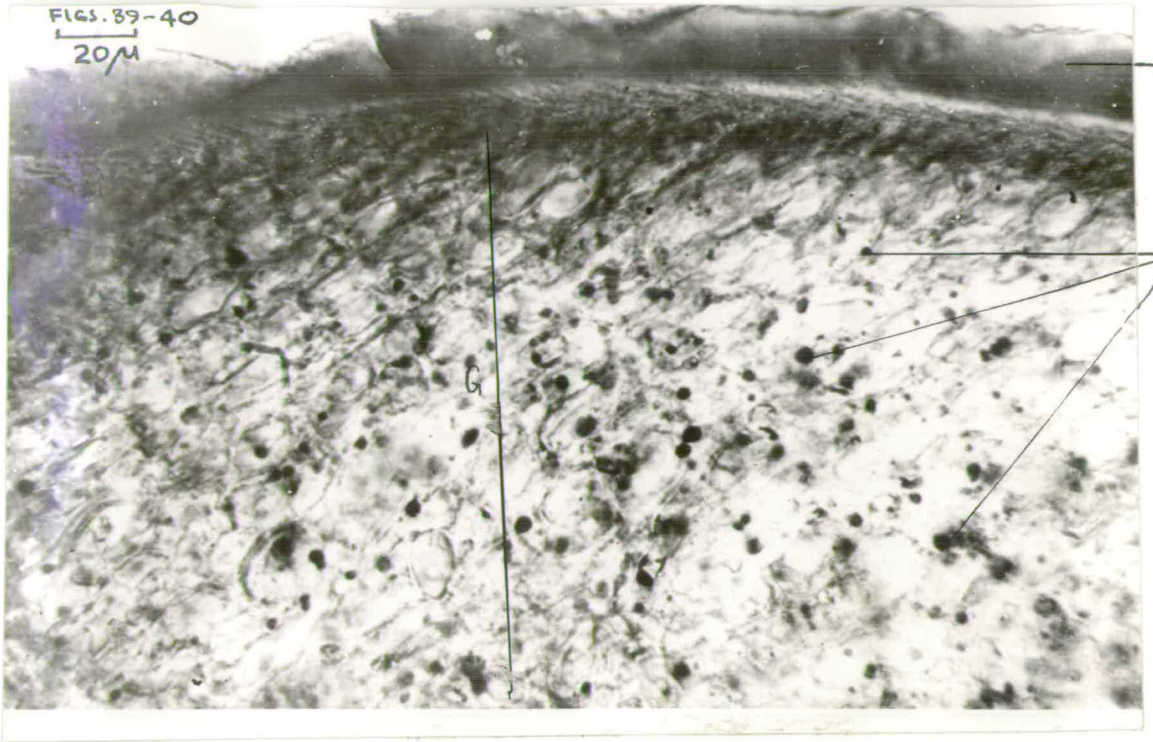

FIG 39

Fig.39. C. tenuicollis. Section of head. Formol calcium frozen section - Sudan Black B (x 1/6 dry). Showing sudanophilia in cuticle (A) and sundanophilic globules (U) in parenchyma (G).

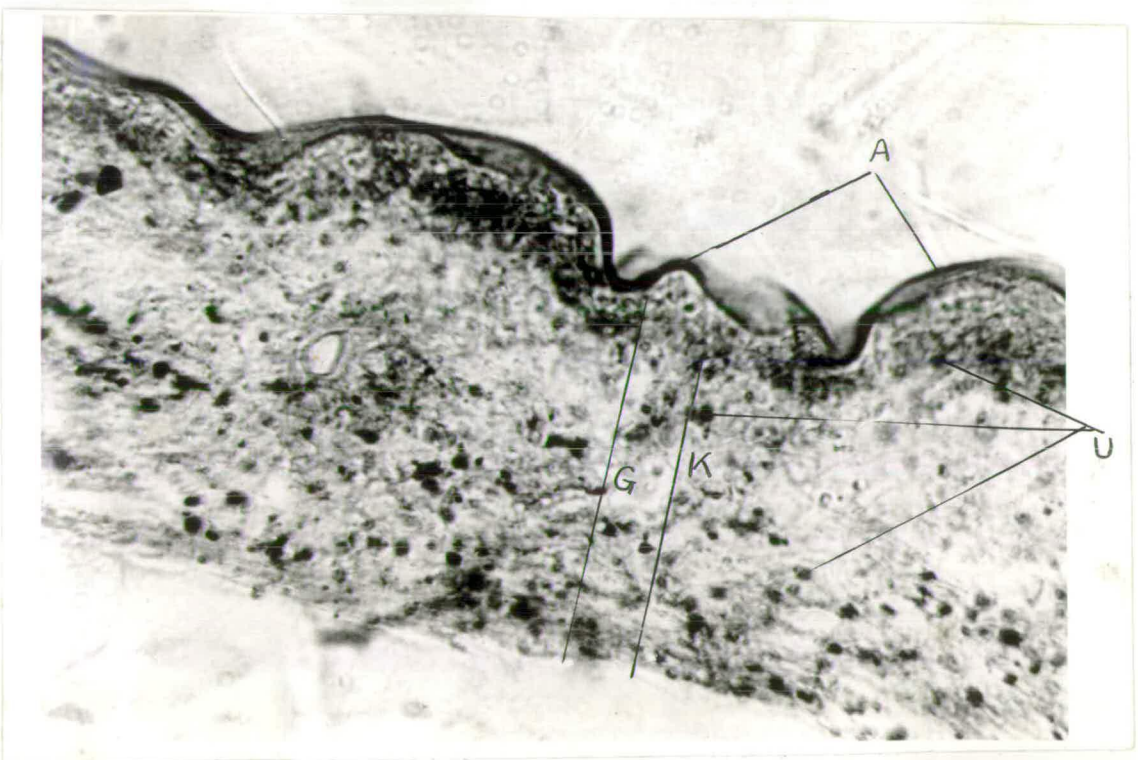

FIG 40

Fig.40. C. tenuicollis. Section of bladder. Formol calcium frozen section - Sudan Black B (x 1/6 dry) showing sudanophilia in cuticle (A) and sudanophilic globules (U) in parenchyma (G).

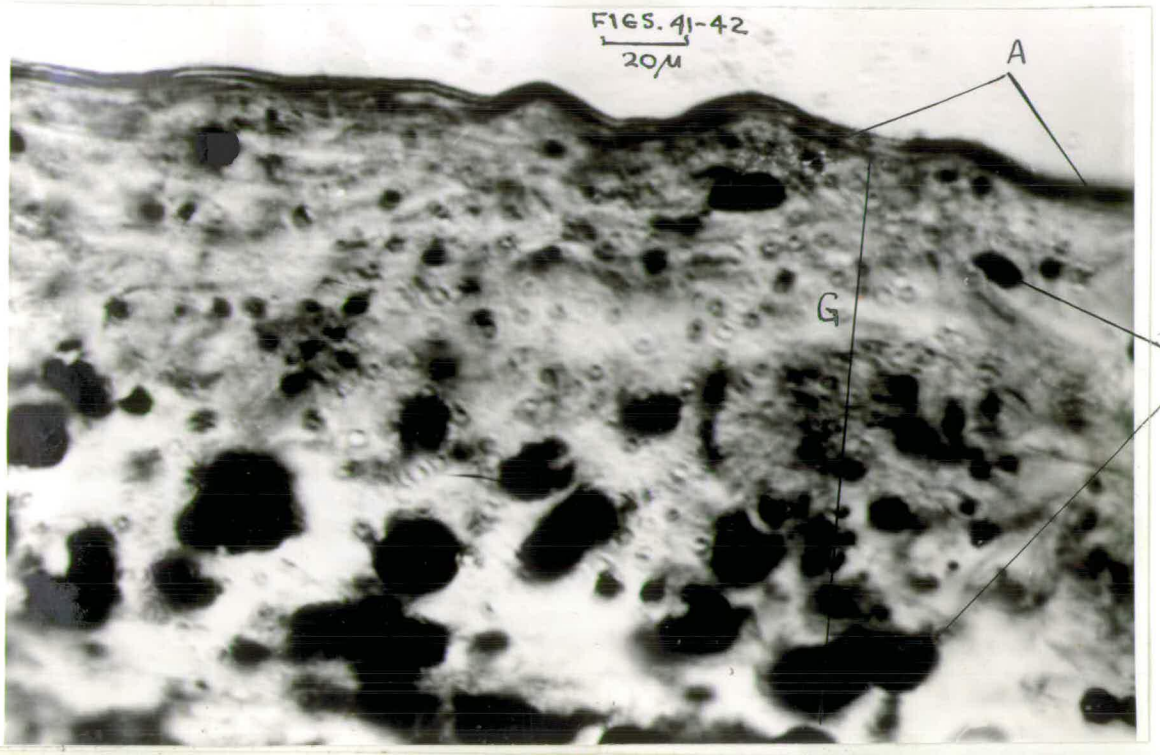

FIG 41

Fig.41. C. tenuicollis. Section of bladder. Formol calcium frozen section - Sudan Black B (x 1/6 dry) showing irregular sudanophilia in cuticle (A) and large sudanophilic globules (U) in parenchyma (G).

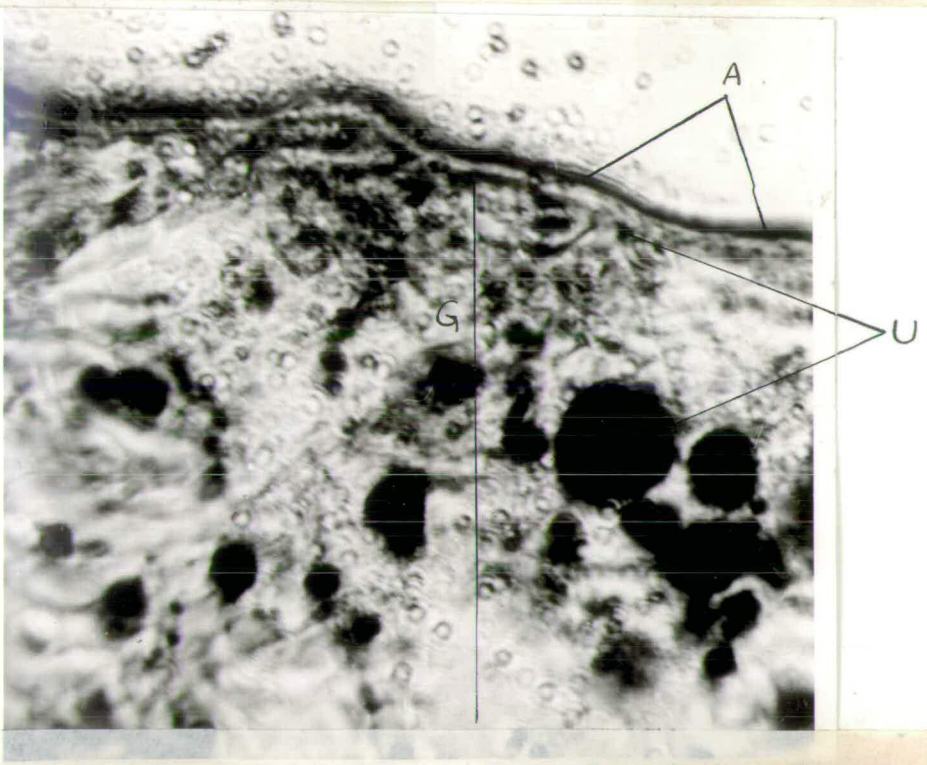

FIG 42

Fig.42. C.bovis. Section of bladder. Formol calcium frozen section - Sudan Black B (x 1/6 dry). Showing irregular sudanophilia in cuticle (A) and large sudanophilic globules (U) in parenchyma (G).
Note. Fixation was accidentally extended to 24 hrs. in the above two figures and compare with normal fixation in figs. 37 to 40.

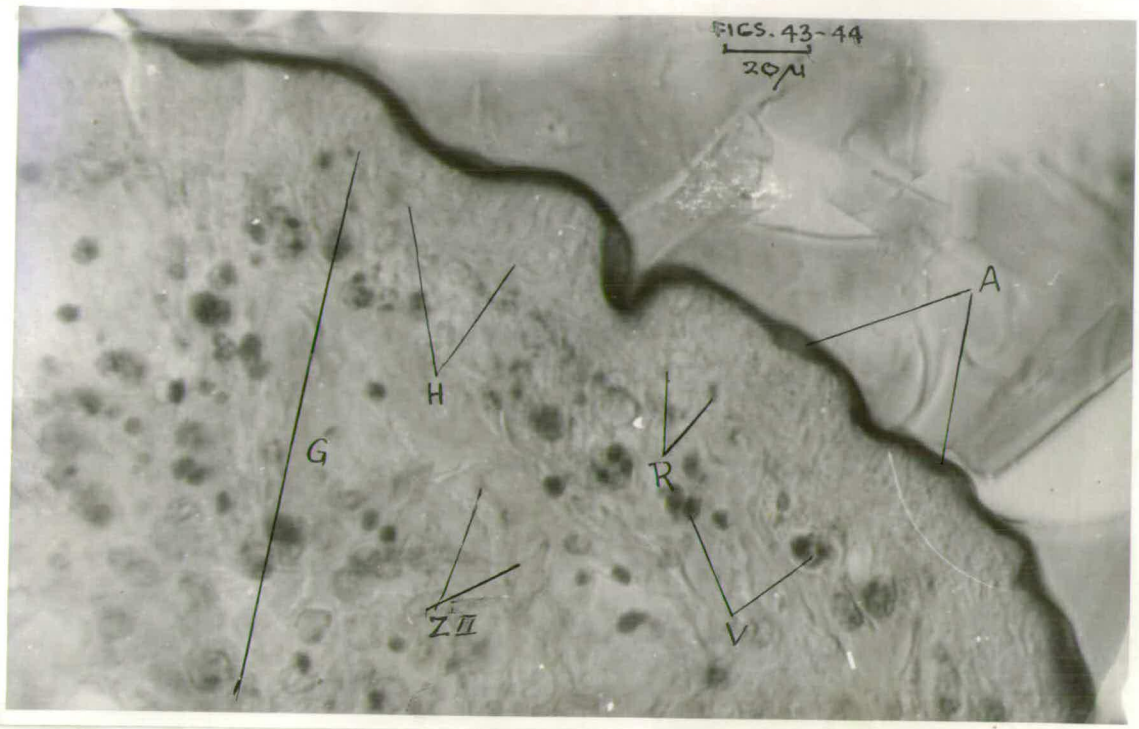

FIG 43

Fig.43. C. bovis. Section of bladder. Formol calcium post chromed - frozen section - acid haematein (x 1/6 dry). Showing positive acid haematein dark colour in cuticle (A) and globules (V) in parenchyma (G).

FIG 44

Fig.44. C. bovis. Section of bladder. Weak Bouin pyridine extracted post chromed - frozen section (x 1/6 dry). Showing absence of dark colour in cuticle (A) and globules (V) in parenchyma (G).

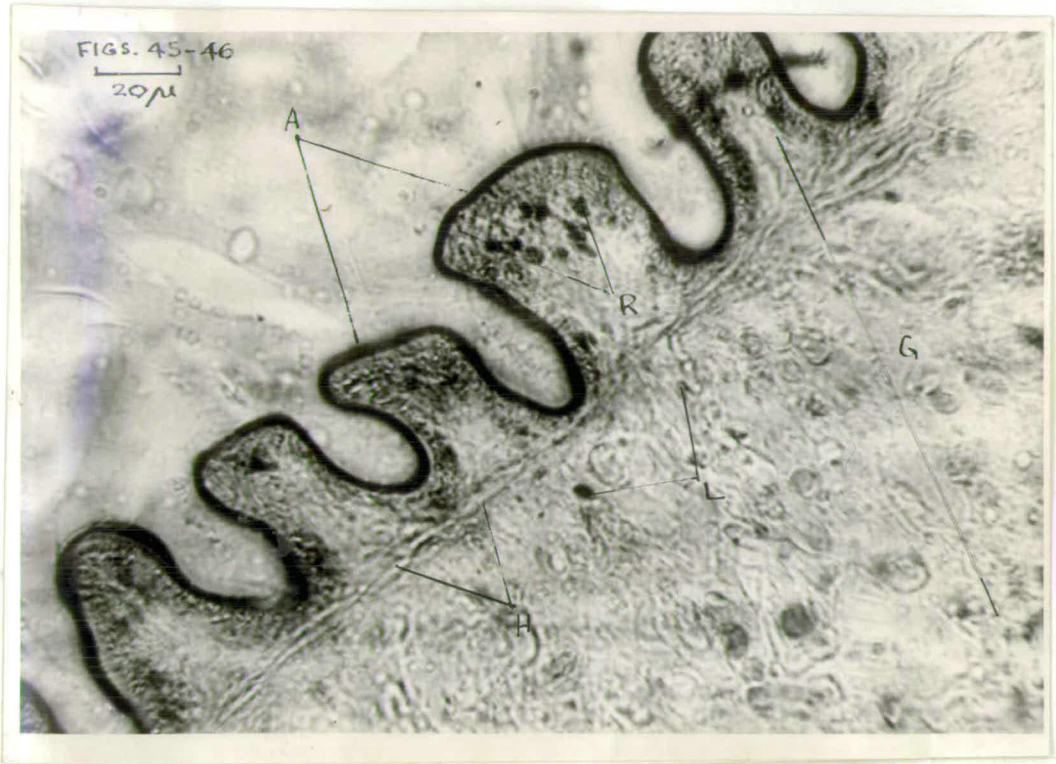

FIG 45

Fig.45. C. tenuicollis. Section of bladder. Formol calcium post chromed - frozen section acid haematein (x 1/6 dry) showing positive acid haematein dark colour in cuticle (A) and globules (V) in parenchyma (G).

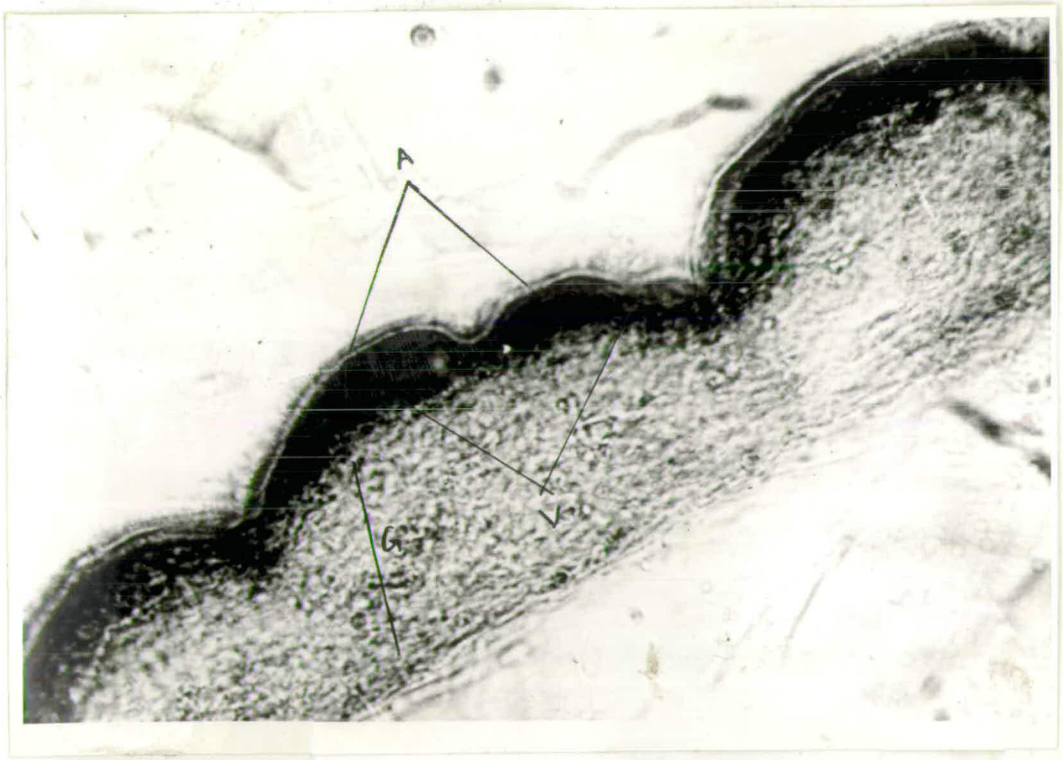

FIG 46

Fig. 46. C. tenuicollis. Section of bladder. Weak Bouin pyridine extracted post chromed frozen section - acid haematein. Showing absence of dark colour in cuticle (A) and globules (V) dislocated in parenchyma (G).

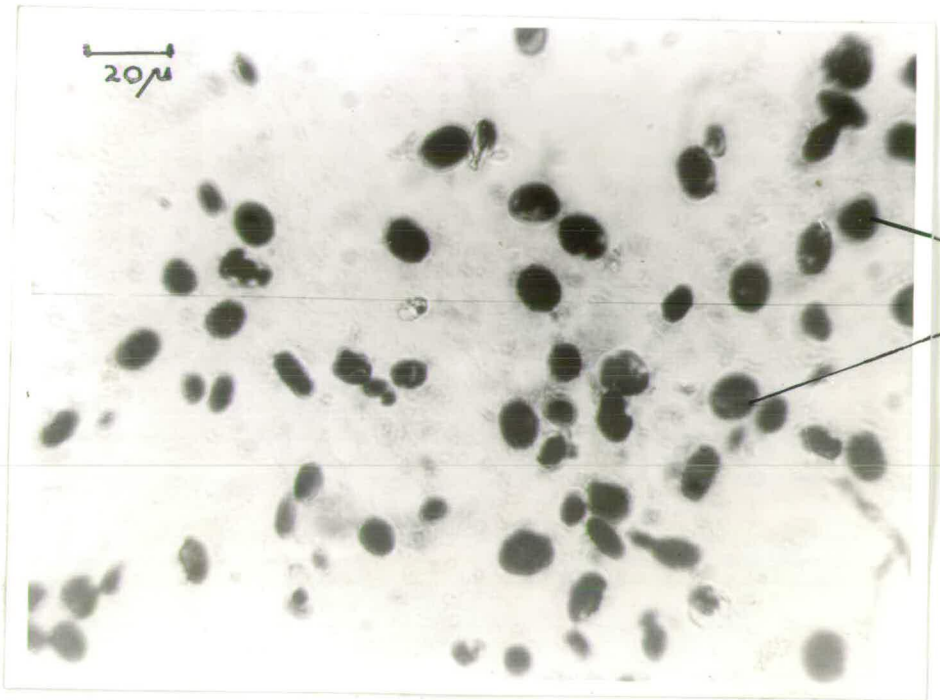

FIG 47

Fig.47. C. bovis. Section of head. Ethanol/acetone - cobalt chloride. Showing presence of calcium (W) in calcareous corpuscles.

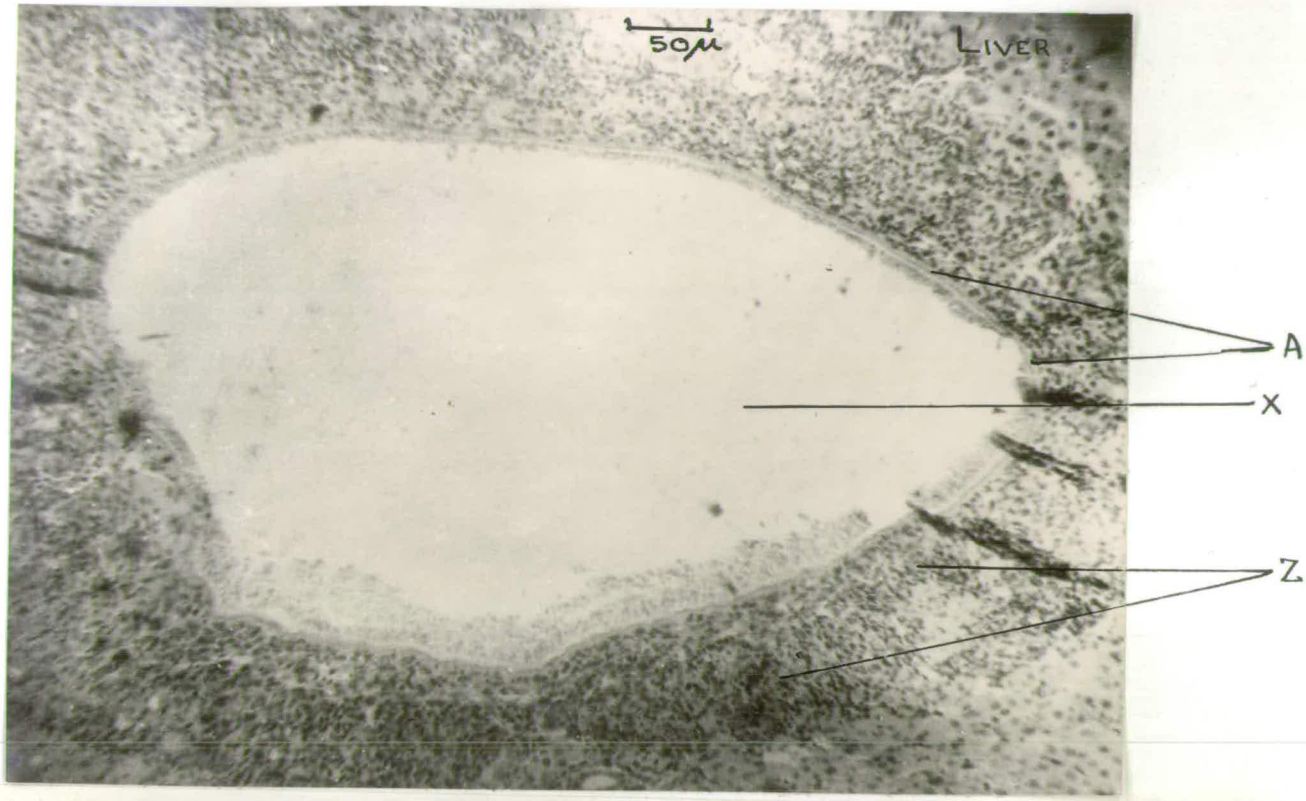

FIG 48

Fig.48. C. fasciolaris. Section of 10 days old cyst. Carnoy - Haematoxylin and eosin (x 2/3). Showing macrophages (2) in pericystic zone around cuticle (A).
Note. Variable thickness of parenchyma (G) of the cyst.

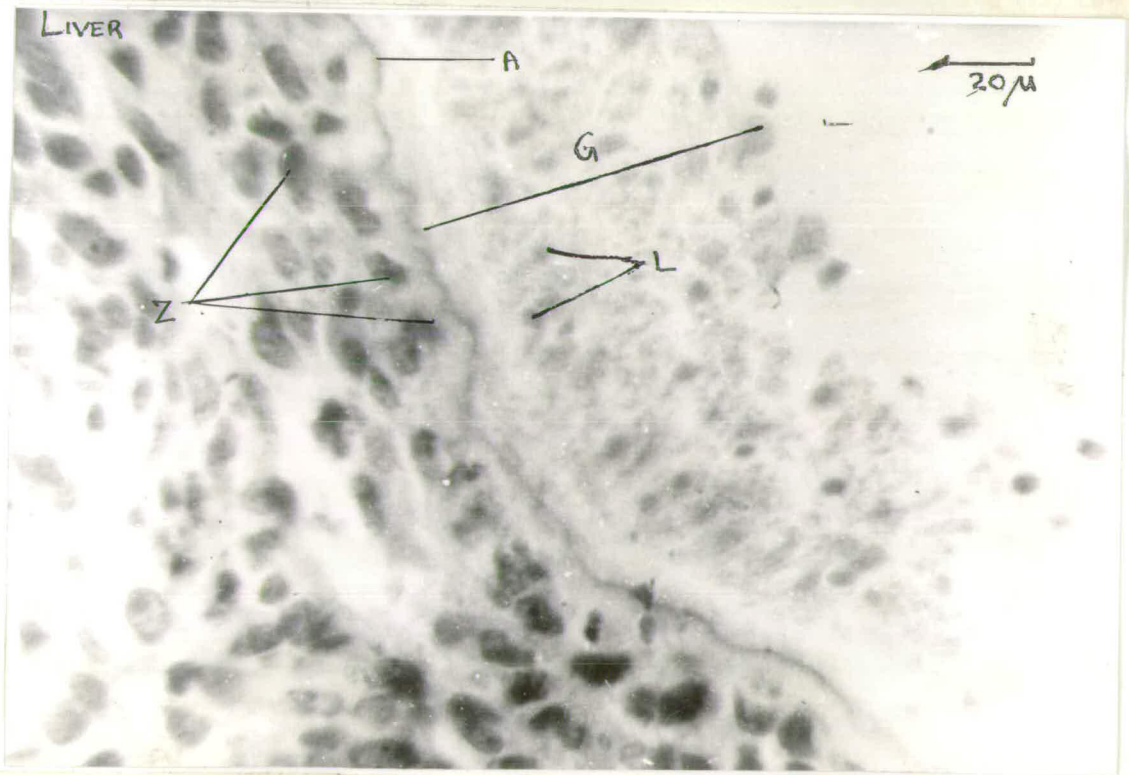

FIG 49

Fig.49. C. fasciolaris. Section of 10 days old cyst. Carnoy - Haematoxylin and eosin (x 1/6 dry). Showing macrophages (2) close to cuticle (A).

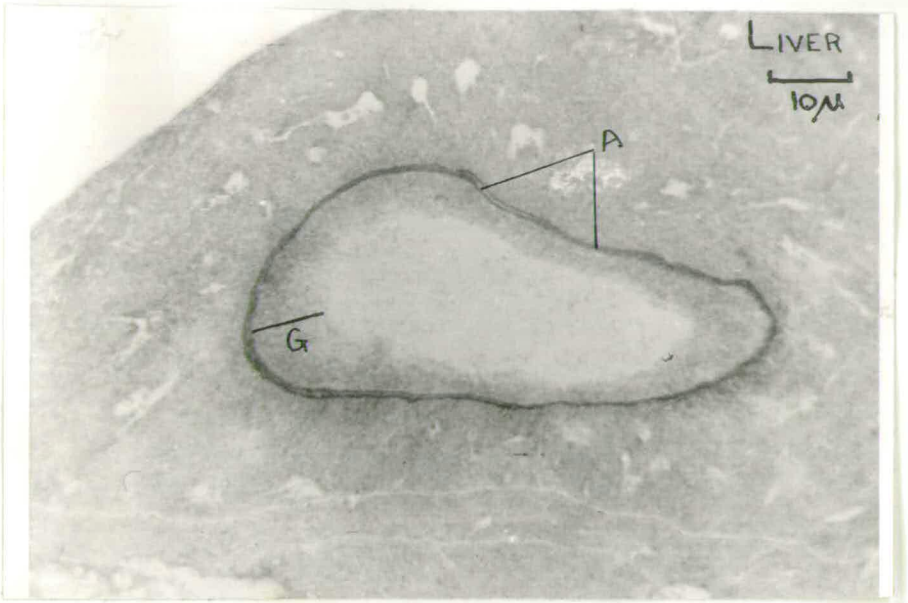

FIG 50

Fig.50. C. fasciolaris. Section of 10 days old cyst. Carnoy - Mallory (x 50). Showing splitting of cuticle (A).

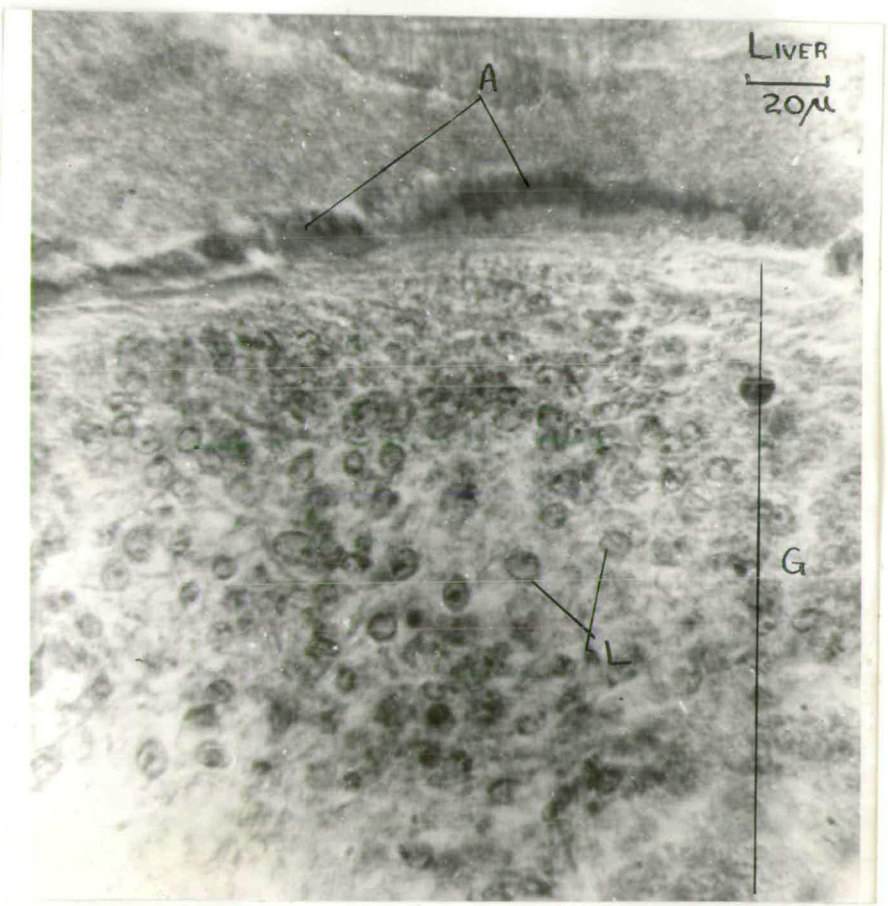

FIG 51

Fig.51. C. fasciolaris. Section of 10 days old cyst. Carnoy - Mallory (x 1/6 dry). Showing projection of cuticle (A) in liver tissues.

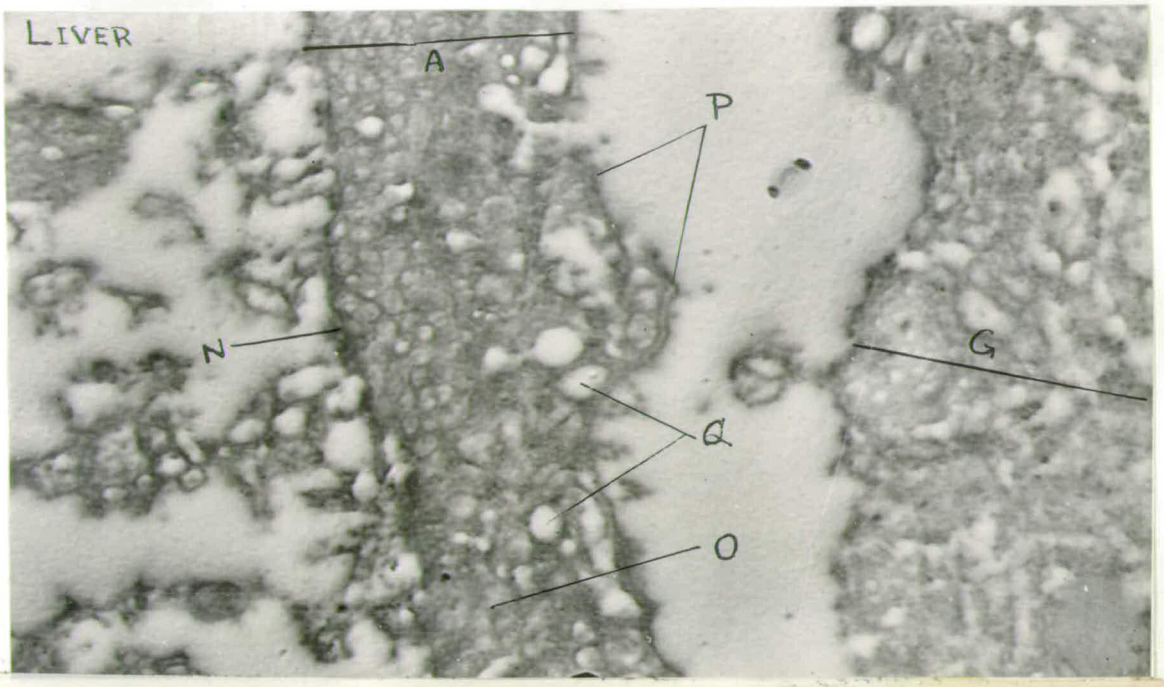

FIG 52

Fig.52. C. fasciolaris. Section of 10 days old cyst fixed Osmium. Electron micrograph (x 13000). Showing structure of cuticle (A).

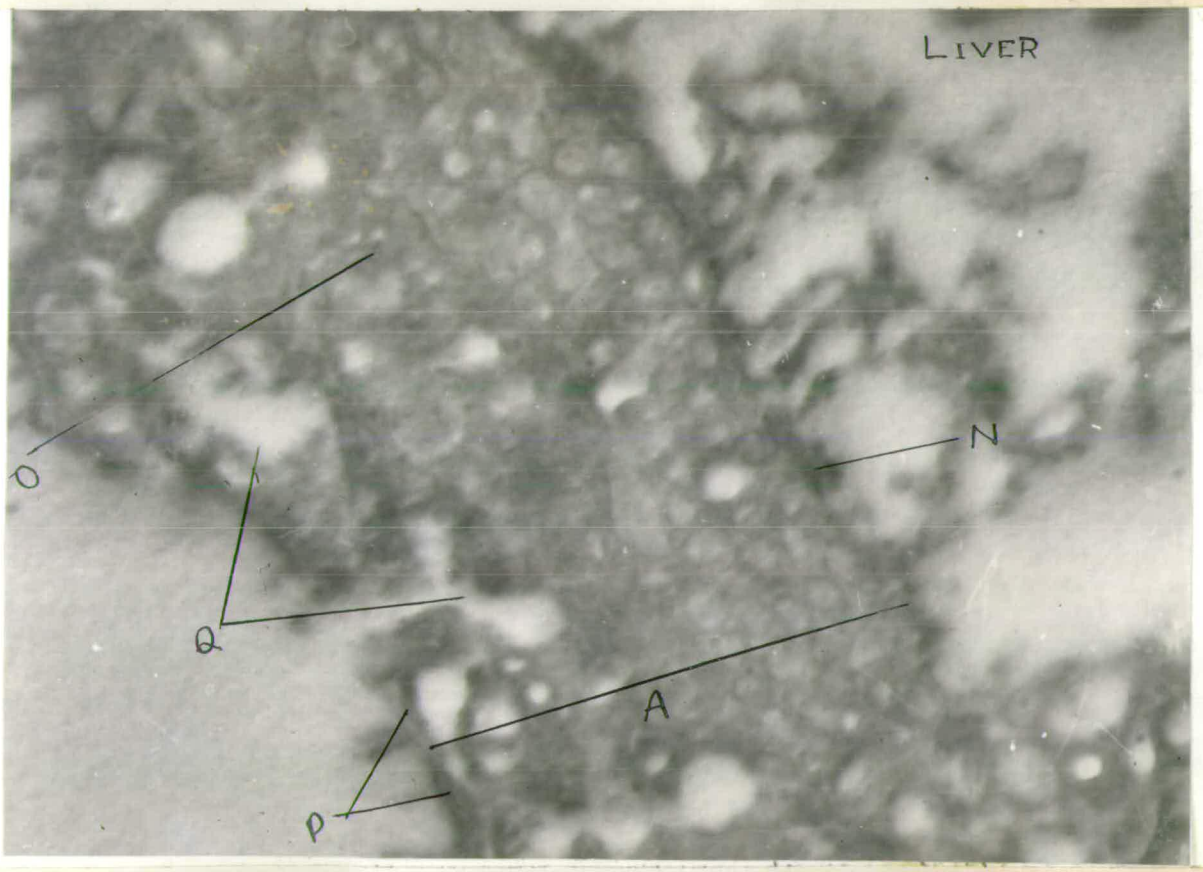

FIG 53

Fig.53. C. fasciolaris. Section of 10 days old cyst fixed Osmium. Electron micrograph (x 26,000). Showing structure of cuticle (A).

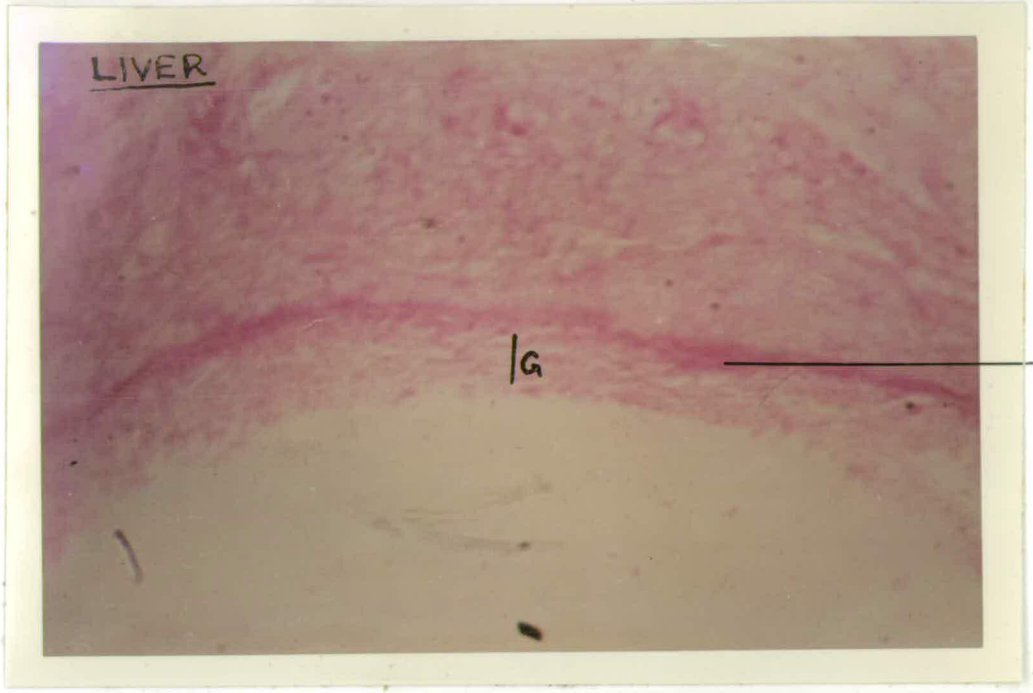

FIG 54

Fig.54. C. fasciolaris. Section of 10 days old cyst fixed Carnoy - PA/S (x 1/6 dry). Showing PA/S positive magenta red colour in cuticle (A) and in parenchyma (G).

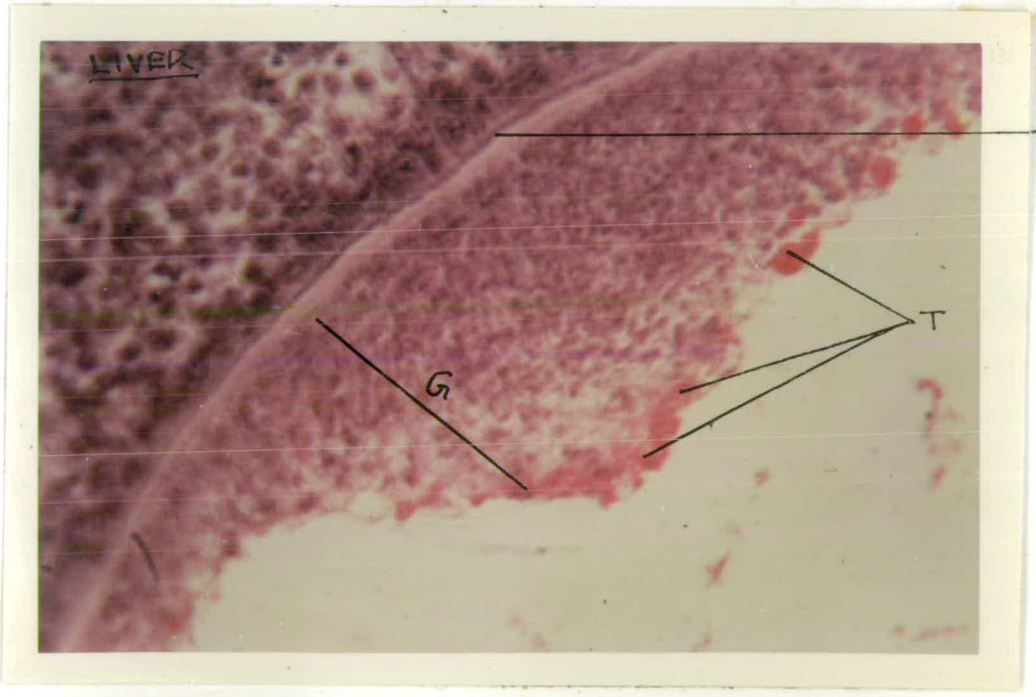

FIG 55

Fig.55. C. fasciolaris. Section of 10 days old cyst fixed Carnoy - Best's carmine - Haematoxylin (x 1/6 dry).
Note. Glycogen granules (T) in parenchyma (G).

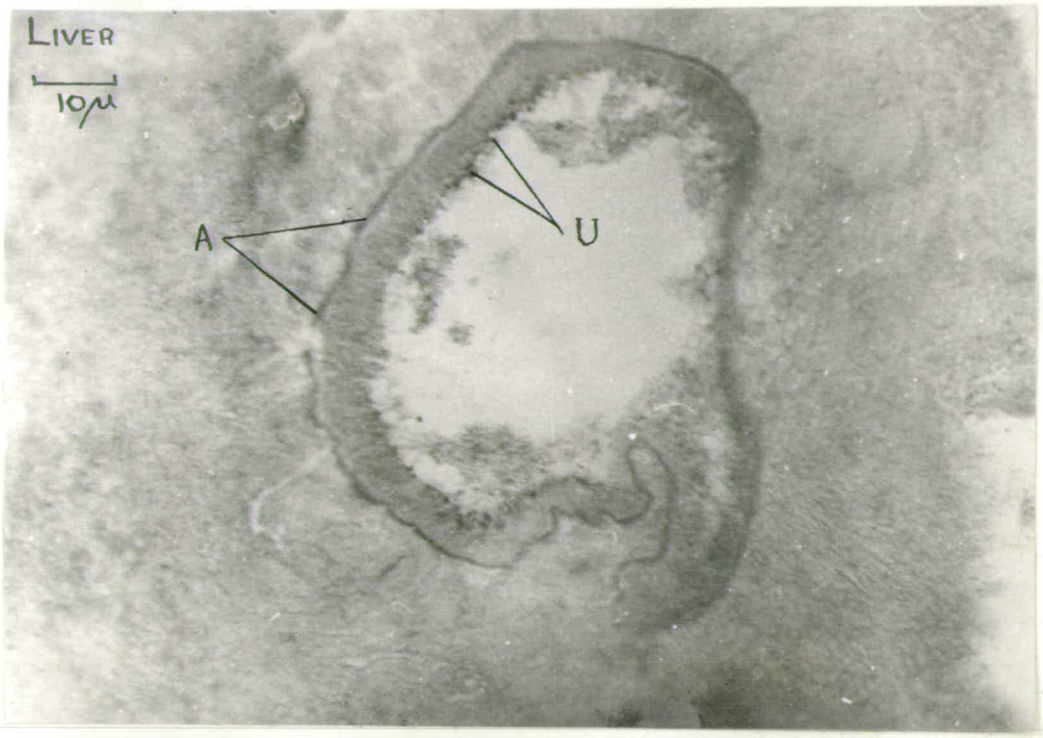

FIG 56

Fig.56. C. fasciolaris. Section of 10 days old cyst. Formol calcium - frozen section - Sudan Black B (x 2/3). Showing sudanophilia in cuticle (A) and sudanophilic globules (U) in parenchyma (G).

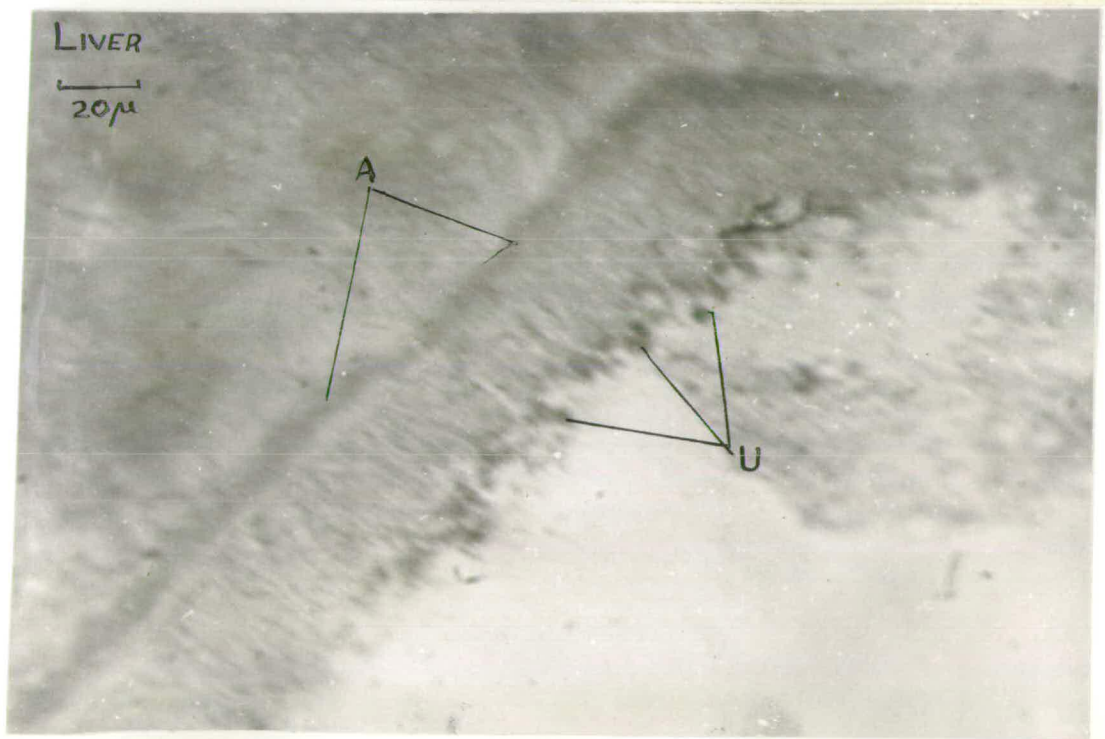

FIG 57

Fig.57. C. fasciolaris. Section of 10 days old cyst. Formol calcium - frozen section - Sudan Black B (x 1/6 dry). Sudanophilia in cuticle (A) and sudanophilic globules (U) in parenchyma (G).

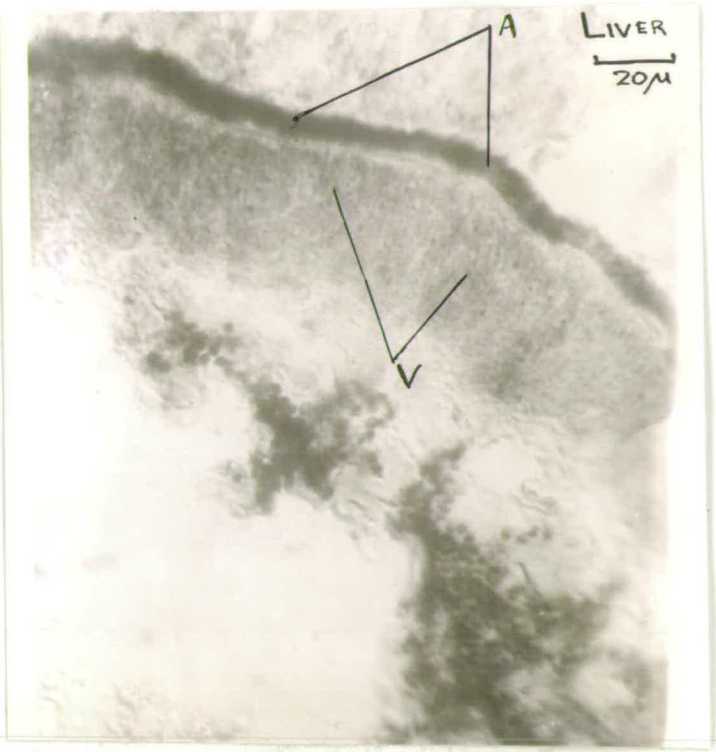

FIG 58

Fig.58. C. fasciolaris. Section of 10 days old cyst. Formol calcium - post chromed - frozen section - acid haematein (x 1/6 dry). Showing dark positive acid haematein colour in cuticle (A) and globules (U) in parenchyma (G).

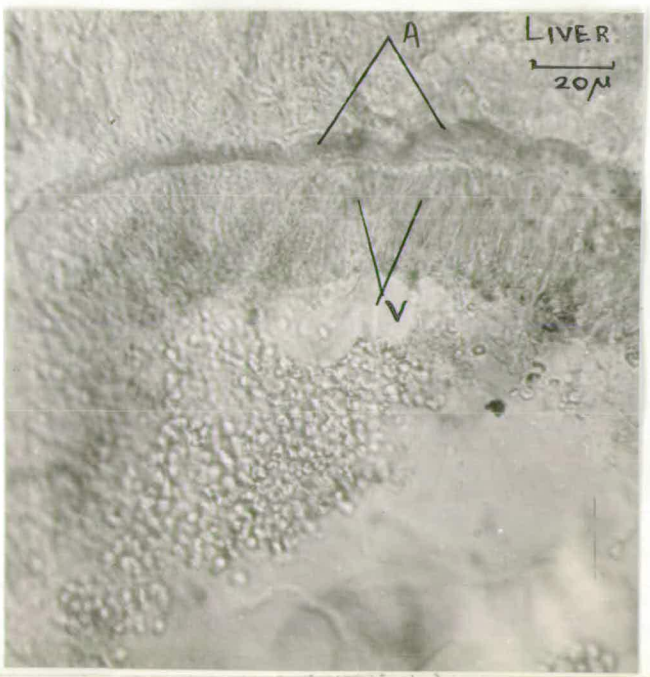

FIG 59

Fig.59. C. fasciolaris. Section of 10 days old cyst. Weak Bouin - pyridine extracted - post chromed - frozen section - acid haematein (x 1/6 dry). Showing absence of dark acid haematein colour in cuticle (A) and absence of globules (U) in parenchyma (G).

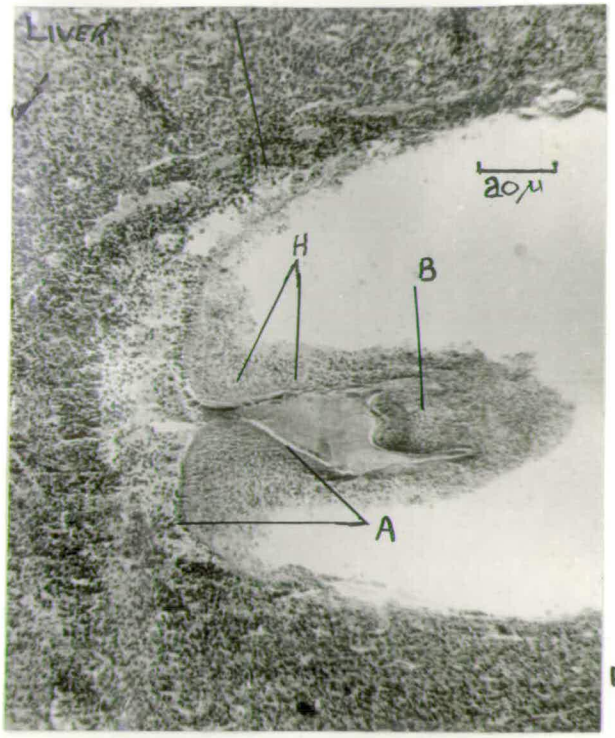

FIG 60

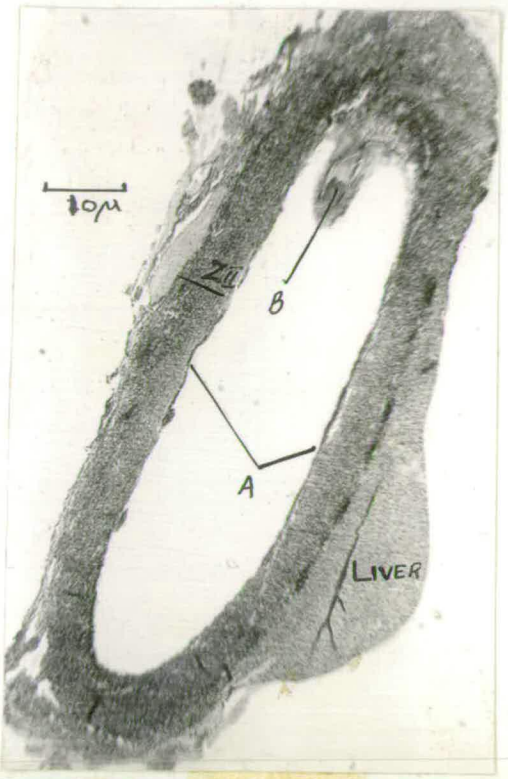

FIG 61

Fig.60. C. fasciolaris. Section of 20 days old cyst. Helly-Mallory (x 1/6 dry). Showing development of head (B) and muscle fibres (H).

Fig.61. C. fasciolaris. Section of 20 days old cyst. Helly-Mallory (x 2/3). Showing complete cyst.
Note. Thickness of cuticle over bladder.

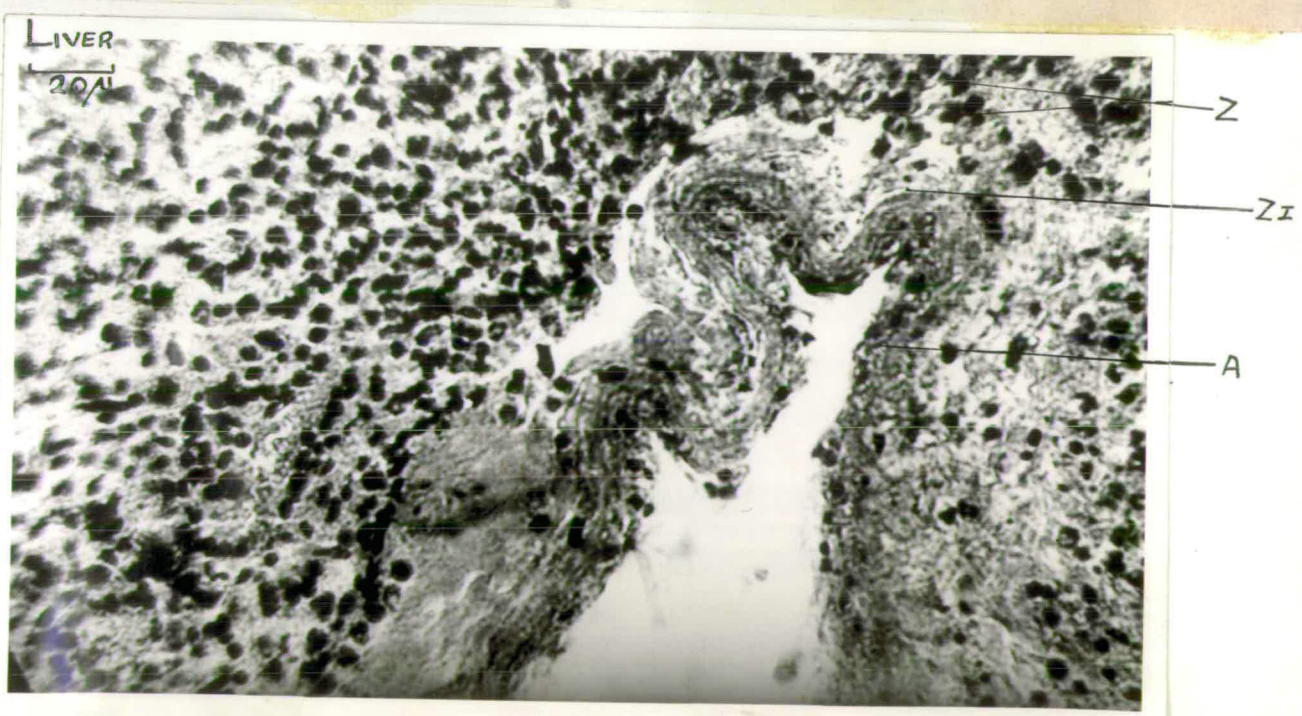

FIG 62

Fig.62. C. fasciolaris. Section of 20 days old cyst. Helly-Haematoxylin and eosin (x 1/6 dry). Showing macrophages (Z) around cuticle (A).

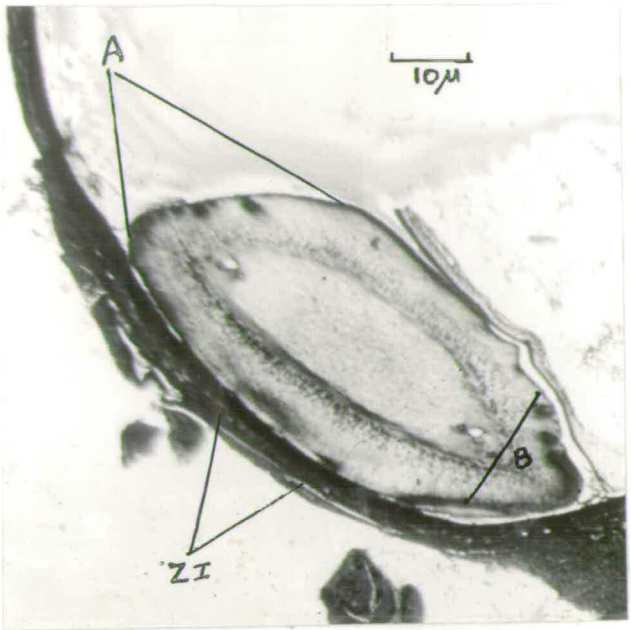

FIG 63

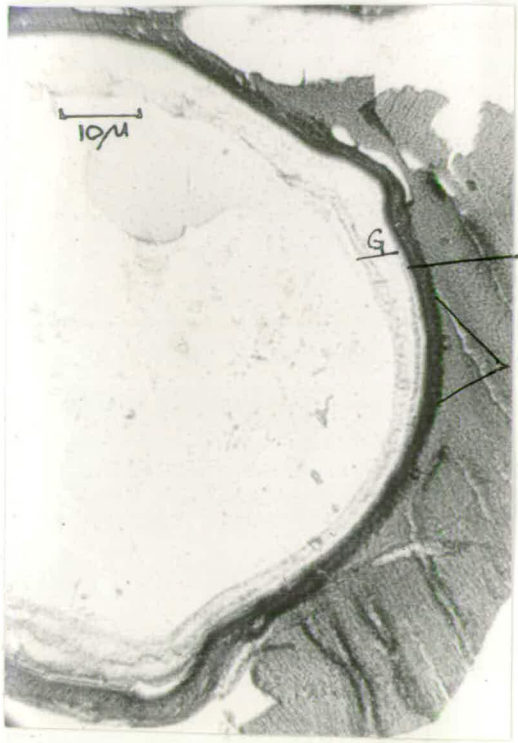

FIG 64

Figs. 63 & 64. C. fasciolaris. Section of 40 days old cyst. Helly-Mallory (x 2/3). Showing connective tissue capsule (ZI) around the cuticle (A) of the cyst.
Note. The development of the head of cyst.

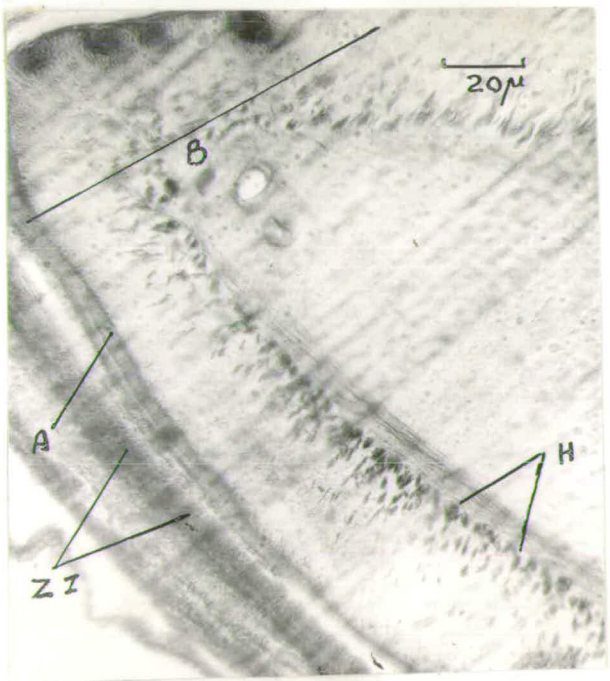

FIG 65

FIG 66

Figs. 65 & 66. C. fasciolaris. Section of 40 days old cyst. Helly-Mallory (x 1/6 dry). Showing development of its head (B) and muscle fibres (H).
Note. Cuticle (A) is attached to connective tissue capsule (ZI).

FIG 67

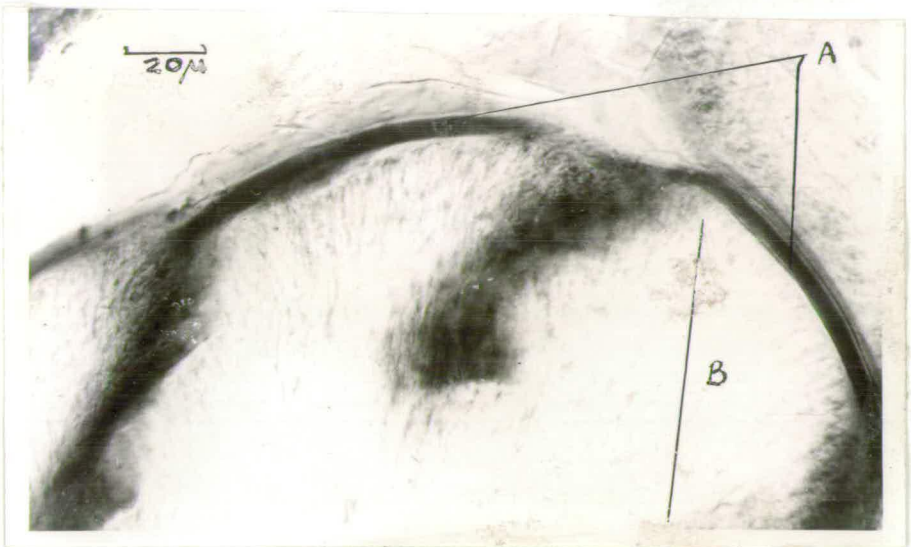

Fig.67. C. fasciolaris. Section of head of 40 days old cyst. Formol calcium - post chromed frozen section - acid haematein. Showing dark colour of acid haematein in cuticle (A).

FIG 68

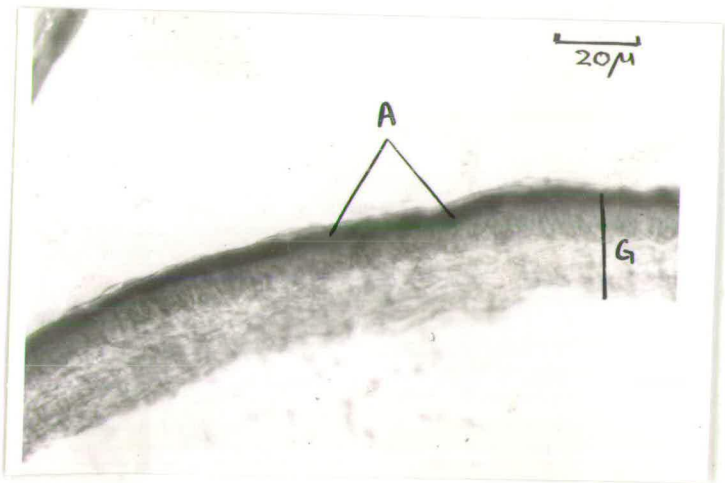

Fig.68. C. fasciolaris. Section of bladder of 40 days cyst. Formol calcium - post chromed frozen section - acid haematein. Showing dark colour of acid haematein in cuticle (A).

FIGS. 69–72.
20μ

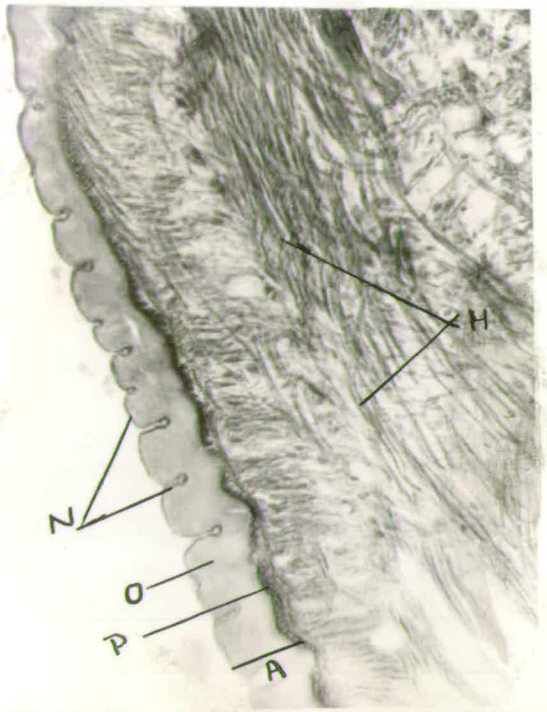

FIG 69

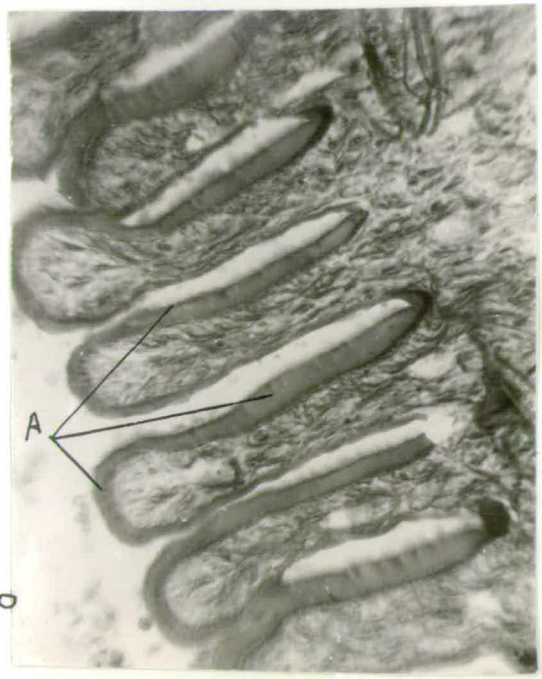

FIG 70

Figs. 69 & 70. C. fasciolaris. Sections of head and strobila region at 60 days old cyst. Helly-Mallory (x 1/6 dry).
Note. In Fig.69 three layers and thickness of cuticle (A) and development of muscle fibres (H). In Fig.70 cuticle (A) is thrown into series of deeply dissected ridges and grooves.

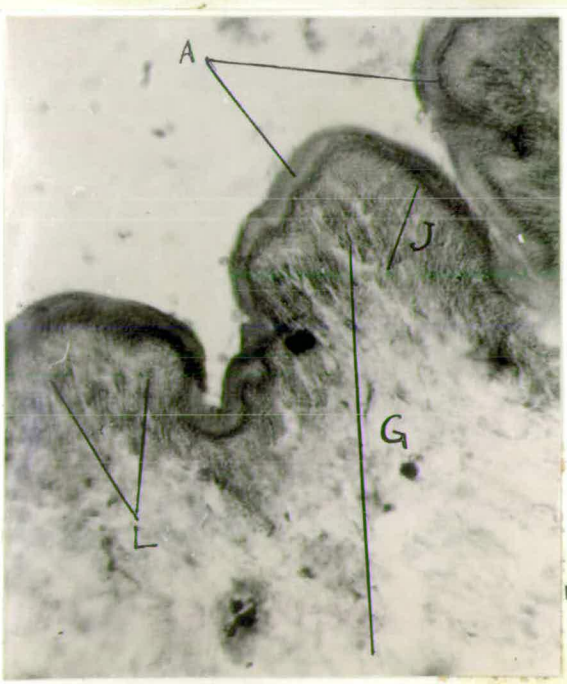

FIG 71

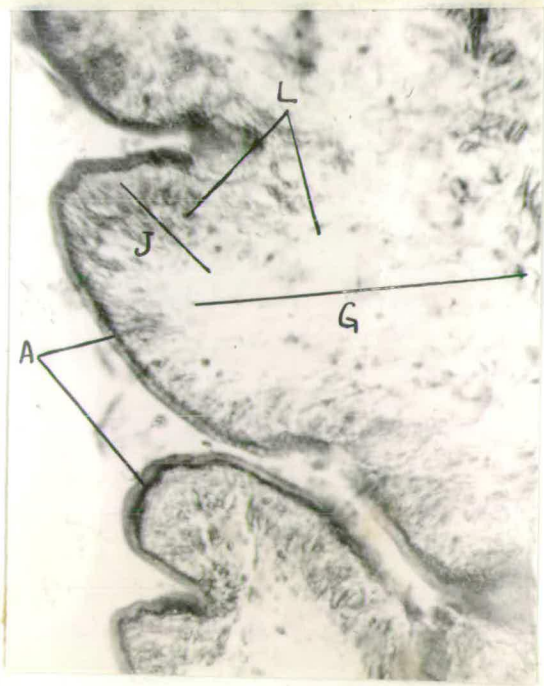

FIG 72

Figs. 71 & 72. C. fasciolaris. Section of bladder of different region at 60 days old cyst. Helly-Mallory (x 1/6 dry).
Note. Variable thickness of cuticle (A) in the two figures.

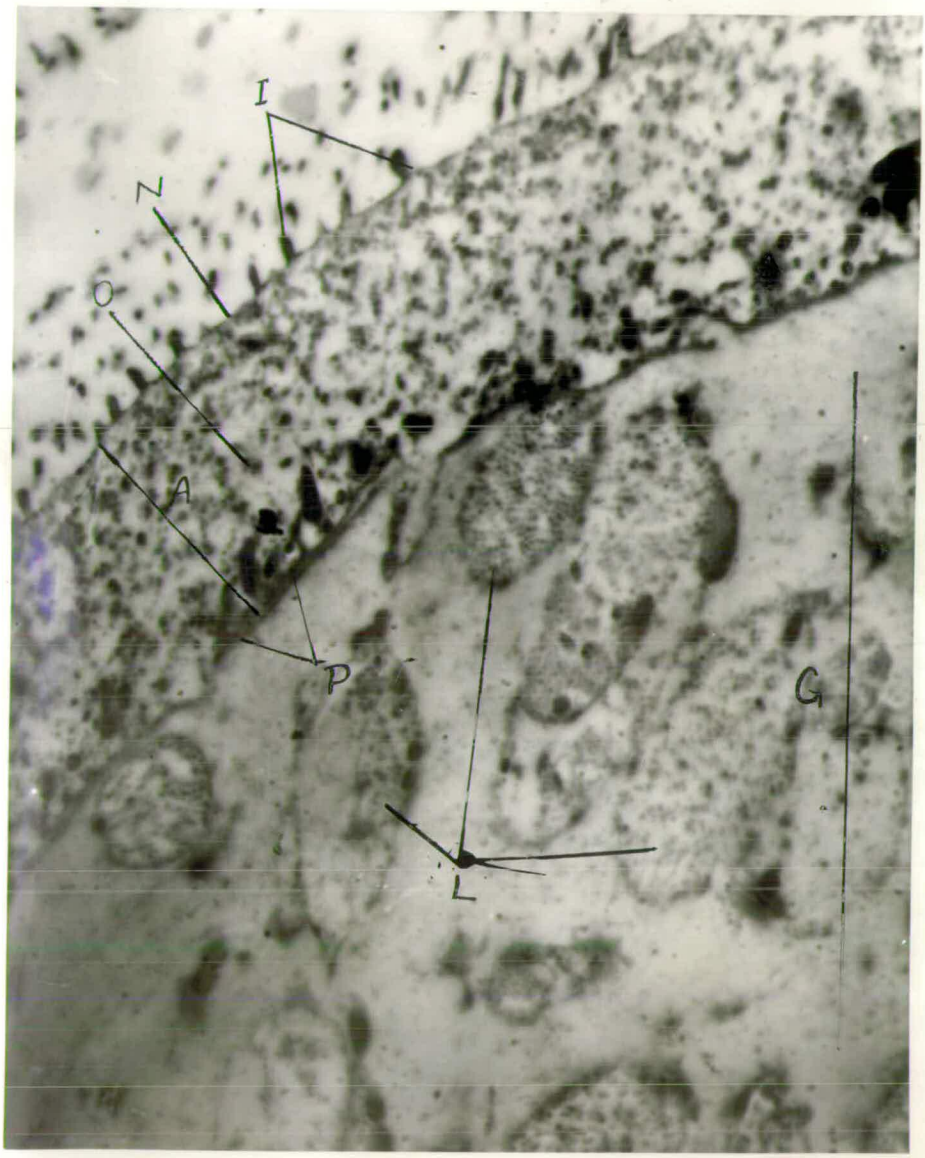

FIG 73

Fig. 73. C. fasciolaris. Section of bladder at 60 days old cyst. Fixed Osmium. Electron micrograph (x 26,000). Showing hairs (i) and three cuticular layers.
Note. Close relationship of the cuticle with nuclei (L) lying in parenchyma (G).

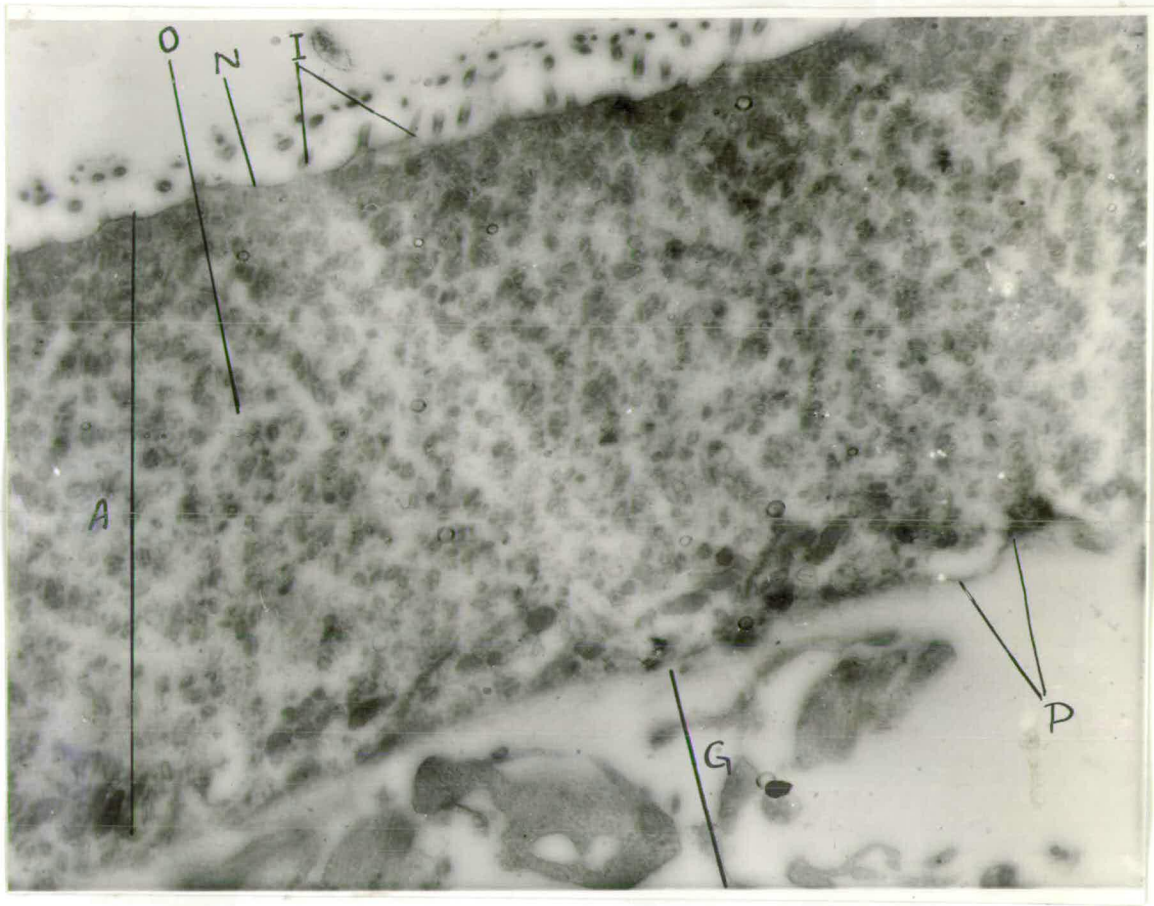

FIG 74

Fig.74. C. fasciolaris. Section of strobila at 60 days old cyst. Fixed Osmium. Electron micrograph (x 26000). Showing structure of cuticle (A).

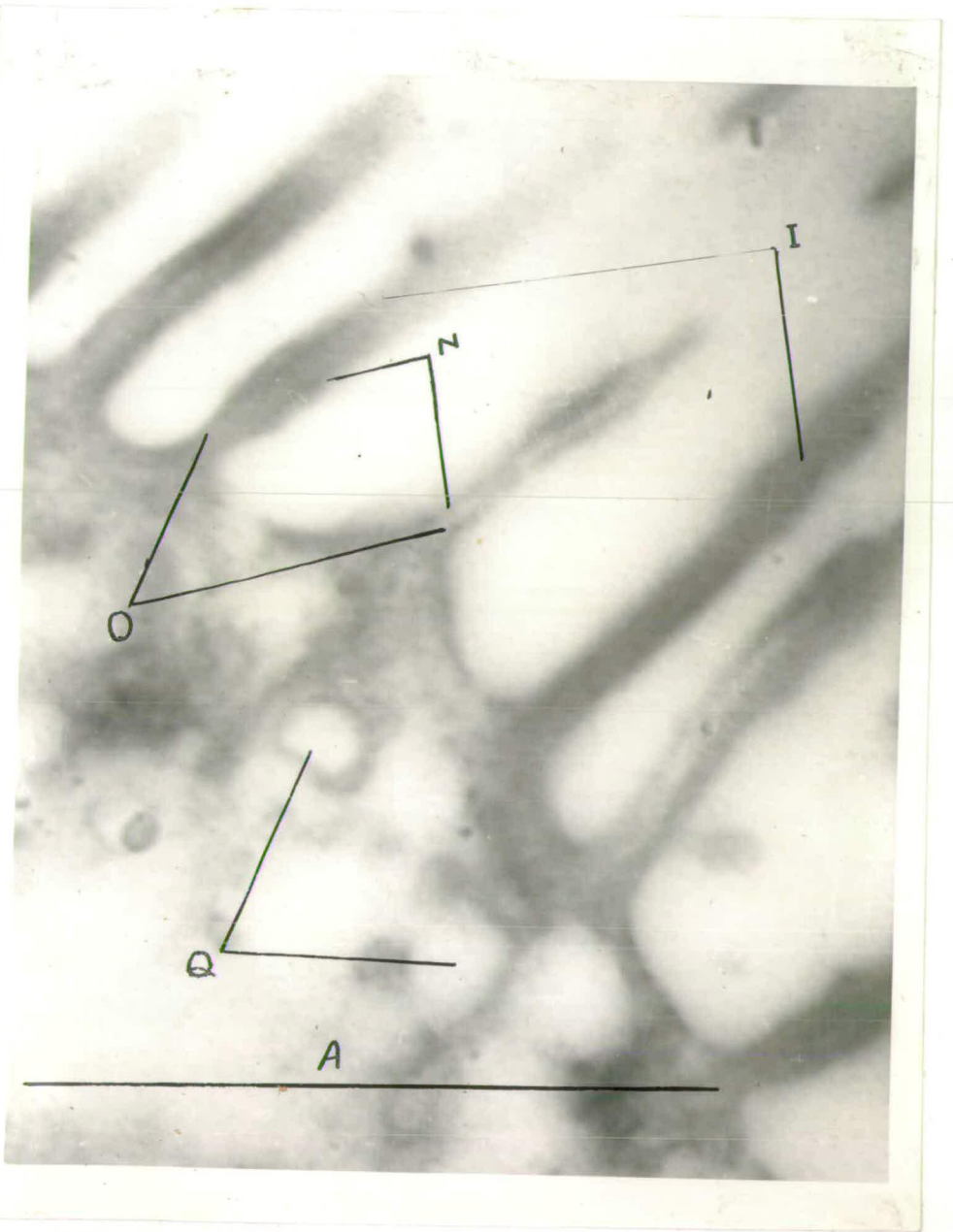

FIG 75

Fig.75. C. fasciolaris. Section of bladder at 60 days old cyst. Fixed Osmium. Electron micrograph (x 80,000). Showing hairs (i) of the cuticle (A).

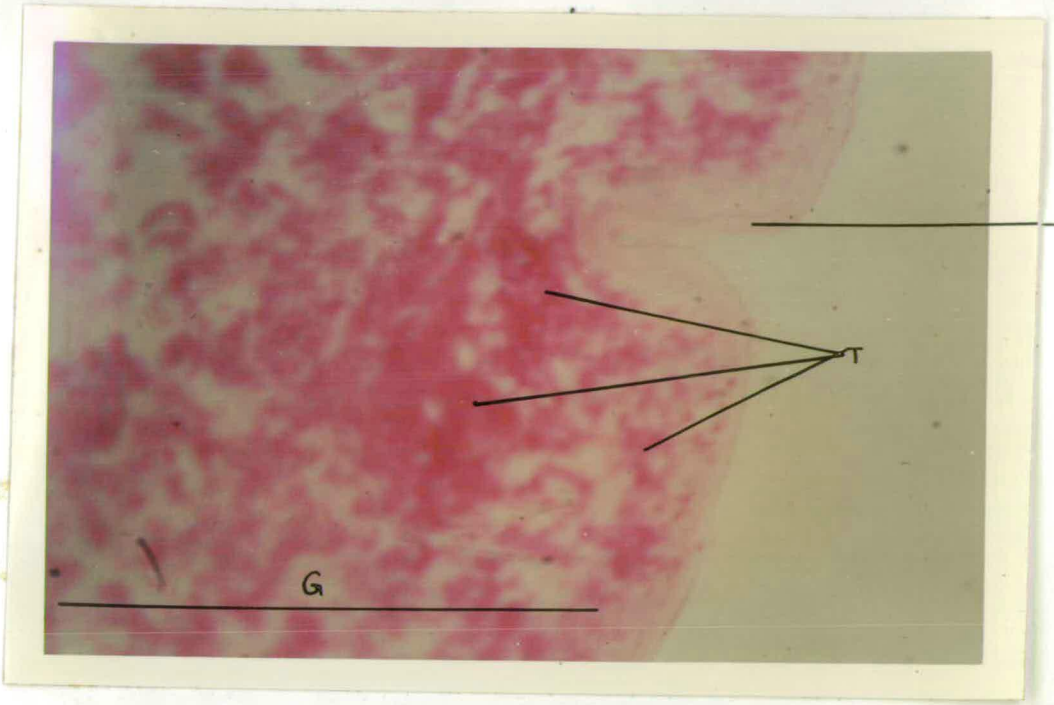

FIG 76

Fig.76. C. fasciolaris. Section of bladder at 60 days old cyst. Carnoy - PA/S (x 1/6 dry). Showing PA/S positive cuticle (A) and PA/S positive granules (T) in parenchyma (G).

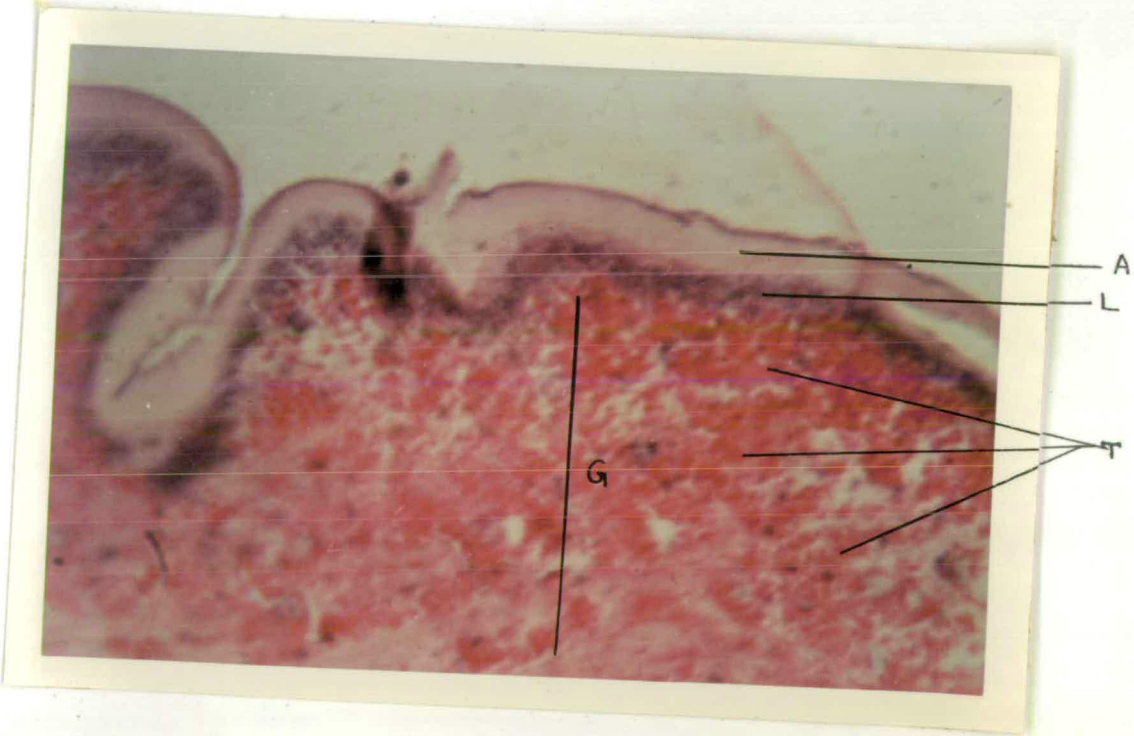

FIG 77

Fig.77. C. fasciolaris. Section of bladder at 60 days old cyst. Carnoy - Best's carmine - Haematoxylin (x 1/6 dry). Showing red glycogen granules (T) in parenchyma (G).

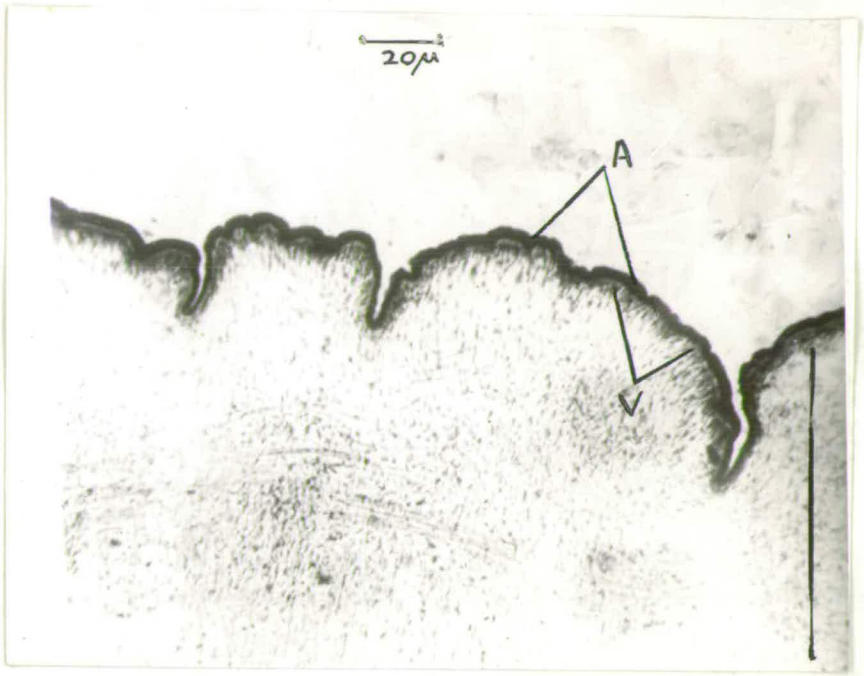

FIG 78

Fig.78. C. fasciolaris. Section of bladder at 60 days old cyst. Formol calcium - post chromed frozen section - acid haematein (x 1/6 dry). Showing dark colour acid haematein in cuticle (A).

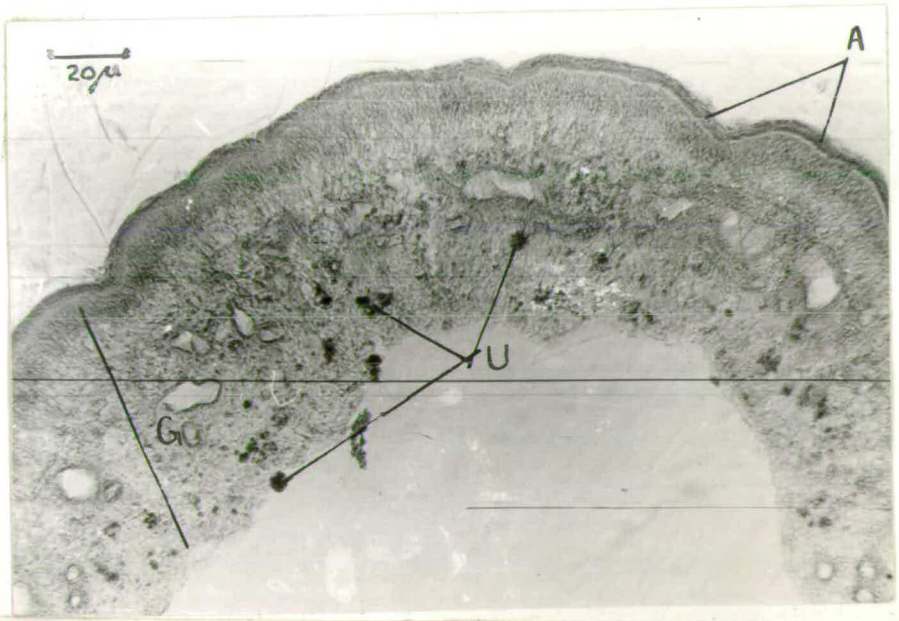

FIG 79

Fig. 79. C. fasciolaris. Section of bladder at 60 days old cyst. Formol calcium - frozen section - Sudan Black B (x 1/6 dry). Showing sudanophilia in cuticle (A) and sudanophilic globules (U) in parenchyma (G).